Tropical bracken, a powerful invader of pastures in South Ecuador: Species composition, ecology, control measures, and pasture restoration

DISSERTATION

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Dedicated to my family

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SUMMARY

Bracken (*Pteridium* spec.) is one of the most wide-spread weeds, especially where fire has been used for forest clearing or maintenance of agricultural areas, in particular of pastures. Taxonomically, it is considered an aggregate that separates into a northern hemispherical and a southern, tropical complex. The taxonomic ranks of the members of these complexes is still a matter of debate. Different from the extensively studied northern bracken, the knowledge of ecology and control measures of the tropical species is still fragmentary.

A research team funded by the German Research Foundation (DFG) is working in an ecosystem study in a narrow valley of the eastern range of the South Ecuadorian Andes. Part of this ecosystem are pastures, replacing the natural rain forest. These pastures are in part heavily infested by bracken and many of them have been already abandoned for this reason. Several groups of the research team work on various aspects of the bracken invasion, e.g. on ecophysiology of bracken, soils and microclimate of the study area, mycorrhiza, and on insect interactions with bracken. The main aims of my thesis were (1) identification and population structure of bracken, (2) ecology of tropical bracken with particular emphasis on its survival of bushfires, and (3) development of an effective bracken control strategy and subsequent re-pasturisation of abandoned areas.

- The bracken vegetation of the study area consists of mixed stands of *Pteridium arachnoideum* (KAULF.) MAXON and *P. caudatum* (L.) MAXON with a proportion of 3:2, and of a hybrid (ca. 2%). Identification was by leaf morphology, allozyme analysis, comparison of particular chloroplastic DNA sequences, and analysis of four genomic and one plastidic microsatellites. Dominance of *P. arachnoideum* was explained by the fact that *P. caudatum*, as a lowland species, reaches its upper altitudinal limit in the research area. Analysis of heterozygosity indicated a higher genetic stability of the diploid *P. arachnoideum* population as compared to the allotetraploid *P. caudatum* population. Spatial extension of the individual clones is apparently much smaller than reported for members of the northern bracken, indicating higher significance of sexual reproduction for the tropical fern in comparison to vegetative propagation by rhizome fragmentation.
- Four weeks after burning the natural rain forest, vigorously sprouting bracken sporophytes were observed. These developed from gametophytes, which

germinated from the wind dispersed spores. Fast growth of the young sporophytes established the fern in the areas. After planting pasture grass, bracken was supported by repeated burning of the areas. In the long run, the grass was outcompeted by the fern possibly due to weakening of its vitality by burning and grazing, and the areas have been abandoned.

- The density of bracken fronds in a settled bracken area of our research site remained constant over years with only small deviations caused by particular weather situations. Since this balance holds also for patchy fern canopies, it is assumed that this is due to nutrient shortage of the soil. Most probably, a new leaf can only develop from the nutrients remobilized from a senescing old leaf.
- Two to three months after a fire, an explosive emergence of new leaves was observed at rates, which substantially exceeded those under undisturbed growth. The newly formed leaves showed an extended life-span, which was attributed to a better nutrient supply from the ash. Subsequent self-thinning reduced the density of the leaves to a stable level within two years.
- In a laboratory experiment, the effects of heat pulse by a simulated bushfire on the bracken rhizomes were investigated. Separated long and short shoots were heated for a short time either in a water bath or embedded in soil. Subsequent to this heat pulse, they were cultivated in original soil. Short shoots showed a significantly higher heat resistance (up to 80°C) than long shoots (up to 60°C). In addition, the short shoots showed elongation growth and an enhanced frond production, whereas long shoots were not stimulated by the heat pulse.
- In a bracken control experiment, thirteen control measures (cutting of the fronds, several herbicides, covering with plastic foil and combinations thereof) were applied over a time-period of 23 months. Each treatment was repeated six times and the effects were recorded monthly. Quarterly cutting of the leaves as well as treatment with a customary herbicide mixture (picloram and metsulforon methyl) were the most effective treatments resulting in a reduction of the standing biomass by 65%. Monthly records of the resprouting bracken was necessary to work out the minimum number of treatments required for a clear control effect. For the five most efficient treatments among two to four applications were necessary. However, complete eradication of bracken was not possible.

- For re-pasturisation, the common pasture grass *Setaria sphacelata* was planted on the treated areas within a long-term experiment. After nearly two years of observation, the system had stabilized with a cover of *S. sphacelata* of 75% and of bracken of below 40%. This result demonstrated that the competitive strength of *S. sphacelata* was sufficient to control bracken once weakened by control treatments.

The long-term experiment and, in addition, an experiment in which a gradually bracken-infested area is subjected to controlled burning, are continued.

ZUSAMMENFASSUNG

Der tropische Adlerfarn, ein extrem invasives Weideunkraut in Südecuador:

Artenspektrum, Ökologie, Bekämpfung und Regeneration des Weidelands

Adlerfarn (*Pteridium* spec.) ist eines der häufigsten Unkräuter mit globaler Verbreitung, besonders auf Flächen, auf denen Feuer zur Rodung von Wald oder als landwirtschaftliche Pflegemaßnahme eingesetzt wurde. Taxonomisch stellt *Pteridium* ein Aggregat dar, das in einen nordhemisphärischen und einen südlichen, tropischen Komplex unterteilt wird. Die taxonomischen Rangstufen der Mitglieder dieser Komplexe sind bisher nicht abschließend geklärt. Im Gegensatz zum gut untersuchten „nördlichen Adlerfarn“ ist das Wissen über die Ökologie sowie über Unkrautbekämpfungsmöglichkeiten der tropischen Vertreter lückenhaft.

Im Untersuchungsgebiet einer von der Deutschen Forschungsgemeinschaft geförderten Forschergruppe in den Ost-Anden Südecuadors vernichtet der Adlerfarn großräumig die dem Bergregenwald durch „Slash and Burn“ abgerungenen Weideflächen. Verschiedene Aspekte dieser Invasion wie z.B. die Ökophysiologie von Adlerfarn, die Böden und das Mikroklima der Flächen, Mykorrhizierung und Insekteninteraktionen werden deshalb von mehreren Forscherteams untersucht. Ziele der vorliegenden Arbeit waren (1) die taxonomische Identifizierung des Adlerfarns, (2) eine Beschreibung seiner Lebensweise unter besonderer Berücksichtigung der Förderung durch das landesübliche Abbrennen der Weideflächen und (3) eine breit angelegte Untersuchung zur Bekämpfung und Restaurierung der verunkrauteten Weideflächen.

- Im Untersuchungsgebiet treten zwei Arten des tropischen Adlerfarns, nämlich *Pteridium arachnoideum* (KAULF.) MAXON und *P. caudatum* (L.) MAXON im Verhältnis von 3:2, sowie ein Hybrid auf (ca. 2%). Dies konnte anhand morphologischer Blatt-Merkmale, sowie durch Allozym-Analysen, Untersuchung von Variationen chloroplastischer DNA-Sequenzen und Analyse von vier genomischen und einem plastidischen Mikrosatelliten eindeutig nachgewiesen werden. Die Dominanz von *P. arachnoideum* wird darauf zurückgeführt, dass die Tieflandart *P. caudatum* im Untersuchungsgebiet ihre obere Verbreitungsgrenze erreicht. Des Weiteren wiesen die Heterozygotiewerte auf eine stabilere

Population des diploiden *P. arachnoideum* im Vergleich zum allo-tetraploiden *P. caudaum* hin. Die Klongröße der untersuchten Arten scheint deutlich kleiner als von nördlichen Adlerfarnarten zu sein. Dies deutet auf eine größere Bedeutung der sexuellen Fortpflanzung im Vergleich zur vegetativen Vermehrung durch Rhizomfragmentierung hin.

- Bereits vier Wochen nach Brandrodung des Bergwaldes wurde auf den vegetationslosen Flächen eine sehr große Anzahl keimender Jungpflanzen des Adlerfarns beobachtet. Diese stammten offensichtlich von Gametophyten, die sich schnell aus den vom Wind herangetragenen Sporen entwickelten. Damit war der Adlerfarn auf diesen Flächen etabliert. Nach dem Anpflanzen des Weidegrases, setzte sich der Farn durch wiederholtes Abbrennen dieser Flächen nach und nach durch. Da das Weidegras möglicherweise nicht nur durch das Abbrennen, sondern auch durch die Beweidung in seiner Vitalität geschwächt wurde, überwucherte der Farn das Gras schließlich in einem Maße, das dann nicht mehr kontrolliert werden konnte und zur Aufgabe der Weideflächen führt.
- In etablierten Farnflächen blieb die Wedeldichte mit geringfügigen wetterbedingten Schwankungen über Jahre konstant. Da dies auch für lückige Bestände gilt, wird angenommen, dass das Wachstum des Licht-liebenden Farns durch die Nährstoffverfügbarkeit limitiert ist.
- Zwei bis drei Monate nach dem Abbrennen wurde eine explosionsartige Neubildung von Farnwedeln beobachtet, die weit über der Rate bei ungestörtem Wachstum lag. Die in dieser Zeit gebildeten Wedel zeigten eine um einen Monat verlängerte Lebenszeit, was mit der durch die Ascheauflage verbesserten Nährstoffsituation zusammenhängen dürfte. Durch Selbstausdünnung wurde im Verlauf von zwei Jahren wieder eine stabile Wedeldichte erreicht.
- In einem Laborexperiment wurden die Effekte einer von einem Buschfeuer ausgehenden Hitzewelle auf die Rhizome untersucht. Isolierte Lang– und Kurztriebe wurden kurzfristig entweder im Wasserbad oder eingebettet in Erde erhitzt. Danach wurden sie wieder ausgepflanzt. Kurztriebe zeigten eine signifikant höhere Hitzeresistenz (bis 80°C) als Langtriebe (bis 60°C). Des Weiteren wurde ihr Längenwachstum sowie die Bildung neuer Wedel signifikant

stimuliert. Langtriebe zeigten hingegen keine nachweisbare Stimulierung durch den Hitzepuls.

- In einem Farnbekämpfungsexperiment wurden 13 Verfahren (Abschneiden der Wedel, verschiedene Herbizide, Abdecken mit Plastikfolie und Kombinationen dieser Behandlungen) über einen Zeitraum von 23 Monaten angewendet. Die Behandlungen wurden sechsmal durchgeführt und deren Effekte monatlich protokolliert. Am effektivsten erwies sich vierteljährliches Abschneiden der Wedel sowie das Ausbringen einer landesüblichen Herbizidmischung aus Picloram und Metsulfuron-Methyl. Beide Methoden führten zu einer Reduktion der oberirdischen Biomasse (Frischgewicht) um 65%. Durch monatliches Protokollieren des Wiederaustreibens des Adlerfarns wurde die Mindestanzahl der jeweiligen Behandlungen ermittelt. Die fünf effektivsten Behandlungen benötigten zwei bis fünf Anwendungen, um ihre maximale Wirksamkeit zu erreichen. Infolge der tief im Boden liegenden Langtriebe, welche der Nährstoffspeicherung dienen, kann man den Adlerfarn auch durch langfristige Bekämpfung nicht völlig ausrotten.
- Man kann ihn jedoch nach entsprechender Schwächung durch Konkurrenten, wie z.B. einem starkwüchsigen Weidegras, unter Kontrolle halten. Dies wurde über ein Langzeitexperiment, welches sich an die Bekämpfungsexperimente anschloss, über einen Zeitraum von bisher nahezu zwei Jahren nachgewiesen. Die Versuchsflächen wurden mit dem in Ecuador üblicherweise genutzten Weidegras *Setaria sphacelata* bepflanzt, welches eine Deckung von 75% erreichte und den Farn auf unter 40% reduzierte. Der Versuch zeigte somit, dass eine Regeneration von Weideflächen möglich ist.

Diese Untersuchungen, sowie ein weiteres Experiment zum kontrollierten Abbrennen einer mit Adlerfarn befallenen Fläche, werden im Rahmen der Forschergruppe fortgesetzt.

CHAPTER 1

Introduction

"The bracken fern is either one of the world's worst weeds or one of the most successful pteridophytes ever, depending on one's point of view"

C. N. Page (1986)

Bracken (*Pteridium* spec.) is a member of the Dennstaedtiaceae, a group of ferns that reach back to the Triassic, about 200 million years b.p.. Fossils of *Pteridium* have been dated back to 55 million years b.p. (Holm *et al.*, 1997). Today, bracken is considered as one of the five most successful plant genera in the world (Harper, 1982). Due to its high genetic plasticity, members of the genus *Pteridium* have adapted to a broad spectrum of environmental conditions and habitats, a process that is still going on and that explains the genus' worldwide abundance (Burge & Kirkwood, 1992).

After a brief discussion of the taxonomy of bracken, a portrait of the plant and its ecology is presented in this chapter, focusing on those traits which make bracken so successful all over the world. Irrespective of the type of biome, bracken responds positively to anthropogenic disturbance of the natural vegetation and due to its morphological and (eco)physiological traits has become one of the worst weeds. This thesis deals with two species of the so-called "southern bracken", a clade whose members inhabit especially the tropics. In contrast to the corresponding analogue, the "northern bracken", the tropical species show an uninterrupted all-year growth and are therefore even more invasive as e.g. *Pteridium aquilinum* s.str. in Central Europe. Motivation for the study was a comparison of two manifestations of a tropical montane ecosystem, namely the original mountain rain forest, and pasture land as its anthropogenic replacement system. This comparison is the major subject of a comprehensive ecosystem study that is funded by the German Research Foundation (DFG) since 1997 in the narrow valley of the San Francisco river in the eastern range of the South Ecuadorian Andes. Since about 40% of the pastures were already invaded and destroyed by bracken, a detailed investigation of that weed was suggested. Aiming at a quantification of ecosystem services, several groups of the

research team investigated and still work on the active and also the abandoned pastures: Soil scientists, hydrologists, climatologists, mycologists and plant and animal scientists. Groups from Hohenheim and Osnabrück in particular investigate Carbon and water relations of bracken. Mycorrhiza studies have been started by a group from Munich. The task of the present work was threefold: (1) identification of the species composition of the invader “bracken”, (2) investigation of its ecology with special emphasis on its “fire tolerance” and (3) designing and testing of control measures for that population of “southern bracken” in order to propose affordable measures for re-pasturisation of the abandoned areas.

BRACKEN AS A COSMOPOLITAN WEED

Taxonomy and distribution

Taxonomic classification of bracken is very complex and still controversial (Tryon, 1941; Burge & Kirkwood, 1992; Marrs & Watt, 2006). The monograph of Tryon (1941) using morphological characters is the basis of approaches for recent taxonomic work. He considered bracken as a monotypic species comprising two subspecies and twelve varieties (today some of them have been lifted to the rank of a subspecies or even species). Tryon's subspecies *aquilinum* encompasses eight "varieties" on the northern hemisphere, and the tropical subspecies *caudatum* comprises four "varieties" on the southern hemisphere.

In his comprehensive study using morphological characters, Tryon (1941) already noticed particular problems in the delimitation of bracken species: i) the lack of generally applicable differentiating characters that could be attributed to a outstanding phenotypic plasticity, and ii) the high potential for hybridization. To date, several approaches including genetic analyses have shown the genetic heterogeneity of the *Pteridium* aggregate, and a generally accepted view as to the number of species in the genus *Pteridium* has not yet been attained (e.g. Lellinger, 1985; Cobb *et al.*, 2005; Page, 1989; 1994; Page & Mill, 1995a; 1995b; Ashcroft & Sheffield, 1999; Thomson, 2000; Thomson & Alonso-Amelot, 2002). According to the checklist of the "Global Biodiversity Information Facility" (GBIF Data Portal, www.gbif.net, 2009-09-24), 22 species of *Pteridium* have been recognized.

An assessment of the taxonomy of bracken was made by Der *et al.* (2009) in their recent revision of the genus *Pteridium* which is based on the analysis of chloroplastic DNA variation. The authors distinguished two major clades: A primarily northern hemispheric (Laurasian/African) clade which includes the following ten subspecies of *P. aquilinum, namely pseudocaudatum*, *latiusculum*, *japonicum*, *pinetorum*, *decompositum*, *pubescens*, *wightianum*, *aquilinum*, *capense*, *centrali-africanum*, and parts of *P. caudatum.* The second clade is primarily southern

hemispheric (Austral/South American) and includes four species: *P. esculentum*, *P. arachnoideum*, *P. semihastatum* and (in part) *P. caudatum*. Two of these species, *P. caudatum* and *P. semihastatum*, are tetraploid taxa with putative progenitors from both, the northern and southern clades (Thomson, 2000; Thomson & Alonso-Amelot, 2002; Der *et al.*, 2009). The above mentioned pronounced tendency of hybridization of overlapping allopatric populations is another adaptive trait of bracken. Hybrids, if fertile, are frequently more vital. Hitherto, only one report of a triploid *aquilinum* hybrid based on chromosome numbers exist from the northern hemisphere ($3n$ = 156; Sheffield *et al.*, 1993). Allopolyploidy is a possibility in ferns for rapid adaptive speciation, resulting also in plants of increased size. Other examples of bracken intermediates are suggested by morphological characters between *latisculum* and *aquilinum* in northern Europe and between *caudatum* and *arachnoideum* in South America (Page, 1976), but they have not been genetically verified as hybrids.

The present study deals with a bracken vegetation in tropical South America. The current opinion assumes two species which inhabit this continent (Tryon & Tryon, 1982; Burge & Kirkwood, 1992; Thomson & Alonso-Amelot, 2002; Der *et al.*, 2009), namely the diploid *P. arachnoideum* (KAULF.) MAXON (Central and South America except the extratropical southern tip) and the allotetraploid *P. caudatum* (L.) MAXON (Central America and South America north of the equator). Therefore, the taxonomic rank of bracken in the research area is on the species level.

Based on Tryon & Tryon (1982), Alonso-Amelot & Rodulfo-Baechler (1996), and Thomson & Alonso-Amelot (2002) a compilation of characters and altitudinal ranges of the two species is shown in Tab. 1-1. All characters are related to the leaves (so-called fronds), because it is assumed that structure and dimensions of the rhizomes are very similar and not useful for differentiation. However, this has never been examined in detail.

Tab. 1-1: Comparison of *Pteridium arachnoideum* and *Pteridium caudatum* (according to Tryon & Tryon, 1982; Alonso-Amelot & Rodulfo-Baechler, 1996; Thomson & Alonso-Amelot, 2002). The main differential character is given in bold.

Character	*P. arachnoideum*	*P. caudatum*
Hairiness on the lower frond surface	always with gnarled trichomes on and between veins (shortend, thickened, bent and tuberculate hairs), middle to dense	hairy on veins, but mostly sparse (generally simple hairs, not tuberculate) or glabrous between veins
Additional free lobes at the midrib	**present**	**absent**
Frond colour	generally darker surface	lighter green colouration
Midvein	flattened to winged	flattened to terete
Guard-cell length	below 34 µm	between 38 and 43 µm
Spore size	ca. 30 µm	between 34 and 40 µm
False indusium	0.1 - 0.3 mm; ca. 48 cells per mm, irregular distribution of cells	0.3 - 0.5 mm; ca. 31 cells per mm, relative regular cell distribution, margins not strongly curled
Biomass	relatively high	smaller
Ploidy	diploid	tetraploid
Altitudinal distribution	between 1500 and 3000 m	between 600 and 2400 m

Due to the high morphological plasticity of bracken, only one suitable morphological character exists for the differentiation between both neotropical species, namely the presence or absence of free lobes on the midribs and between pinnules (Tryon & Tryon, 1982) (Fig. 1-1). As with all morphological characters considerable quantitative variation can not be ruled out. A preliminary investigation of the species composition in the research area using this character was conducted, inspecting more than 1000 frond samples from seven sites. According to that record, *P. arachnoideum* was the dominant species (84%), while 15% of the samples were tentatively identified as *P. caudatum*. In addition, putative intermediates between the two species were found in 1% of the samples. As both species grow in mixed stands, species differentiation was not possible in the ecological experiments of this thesis (Chapter 5 and 6).

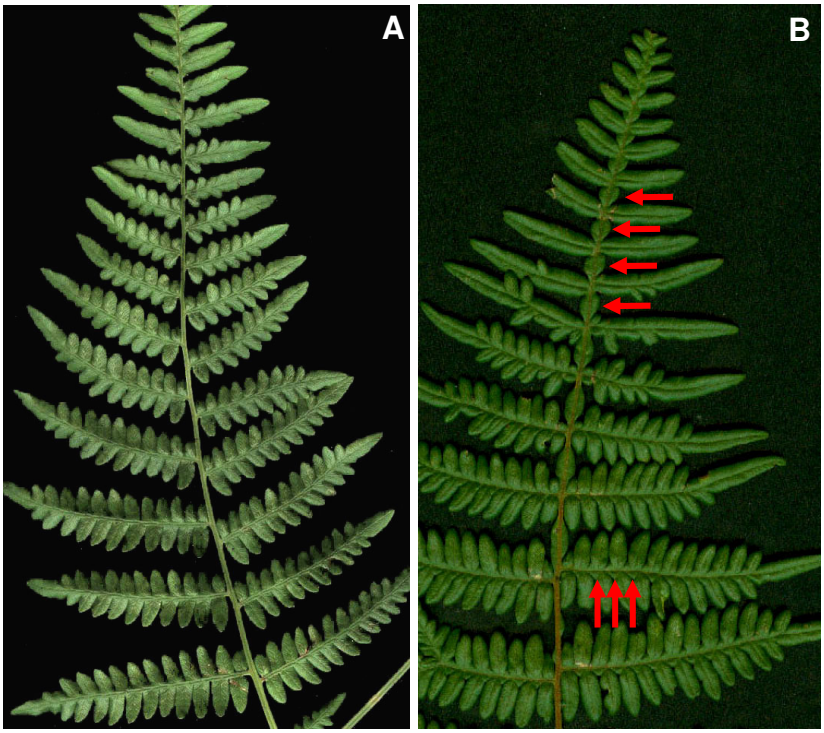

Fig. 1-1: Morphospecies of bracken in the San Francisco Valley, Ecuador A) *Pteridium caudatum* without free lobes, B) *Pteridium arachnoideum* with free lobes on the midrib between segments and pinnules (red arrows).

Population studies of bracken have been performed by allozyme analysis (Speer *et al.*, 1999; Wolf *et al.*, 1988; 1991; Korpelainen, 1995). In addition, phylogeny of bracken has been investigated using the sequences of the chloroplast ribosomal subunit 4 (rps4) and the rps4-trnS intergenic region (Speer, 1999; 2008; Der *et al.*, 2009). But with the latter, clear differentiation between the tropical bracken species *P. arachnoideum* and *P. caudatum* was not possible (Der *et al.*, 2009). A new approach based on microsatellite sequences was recently published in 2008 by Chen *et al.*. Microsatellite analysis is one of the most promising molecular tools to identify and differentiate individuals, varieties and species. The co-dominant inheritance of microsatellites, the high level of polymorphism and reproducibility, and also easy handling make them very useful tools for solving various problems. The availability of this method offered a new possibility for clarifying identity and population structure of bracken. In the present study (Chapter 3), the new developed microsatellites from Chen *et al.* (2008) were used to assess bracken diversity, species composition and clone (genet) extension in the research area.

Biology of bracken as a disastrous weed

Bracken (Fig. 1-2) infests mainly pastures, forested areas, and shrubland, thus causing severe problems for farmers, foresters, and conservationists (Pakeman *et al.*, 1994). Up to now, over 1,600 papers related to bracken have been published. Its importance as a weed is underlined further by five international major conferences since 1985 in UK, Australia, and Portugal.

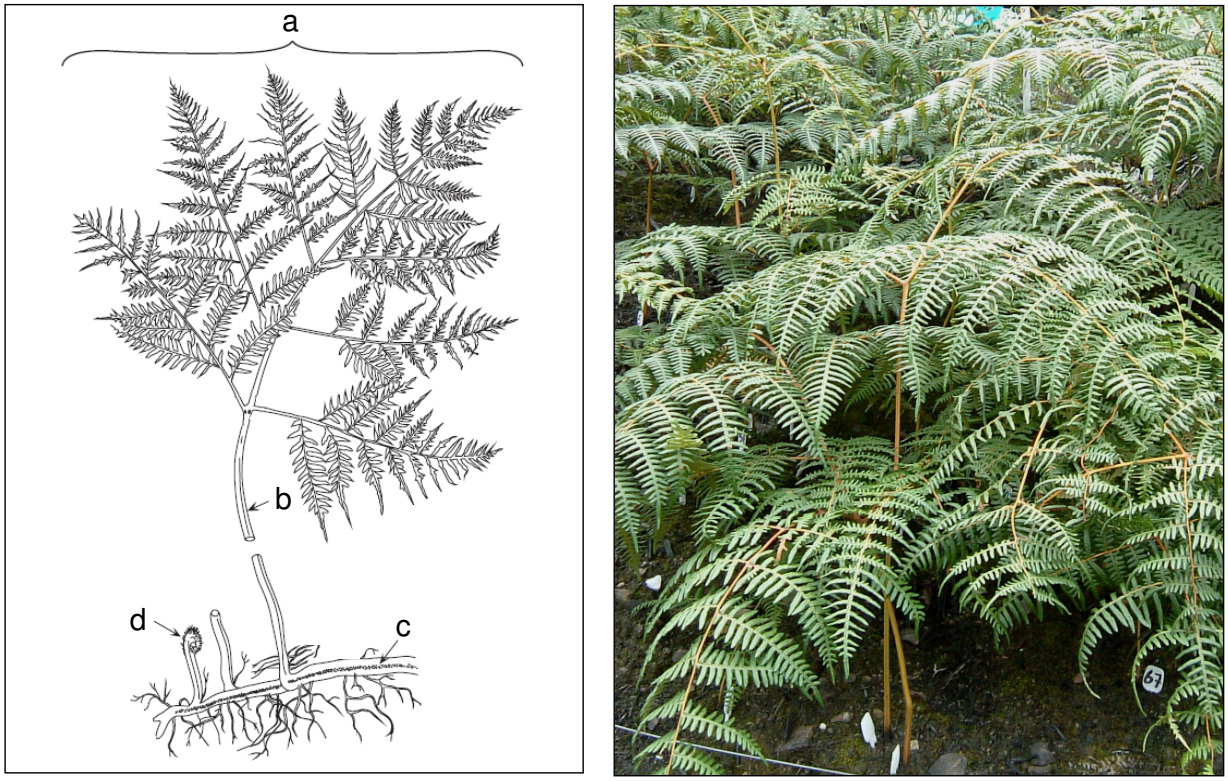

Fig. 1-2: Schematic illustration of european bracken (Marrs & Watt, 2006; modified) and photograph of tropical bracken (K. Roos), (a) frond lamina, (b) stipe, (c) rhizome with (d) crozier.

Main strategies for bracken's success are a high potential for propagation by spores and the long-living rhizomes, high resistance against pathogenic microorganisms, poor palatability for grazing animals, allelopathic effects on competing species, tolerance of weather extremes and bush-fires, and a remarkable genetic plasticity (Page, 1986). Its ***rhizome*** *system* is one of the most important features for the success of bracken (Burge & Kirkwood, 1992). Irrespective of the species, it consists of two components (Fig. 1-3): The fast growing main axis, termed

long shoot, which does not produce fronds, but stores large amounts of starch and prefers deeper soil depths. From this axis the lateral shoots branch off, termed short shoots, which produce the leaves (fronds) and usually concentrate in the top soil. Occasionally, intermediates of both rhizome types have also been found (Watt, 1940; Webster & Steeves, 1958).

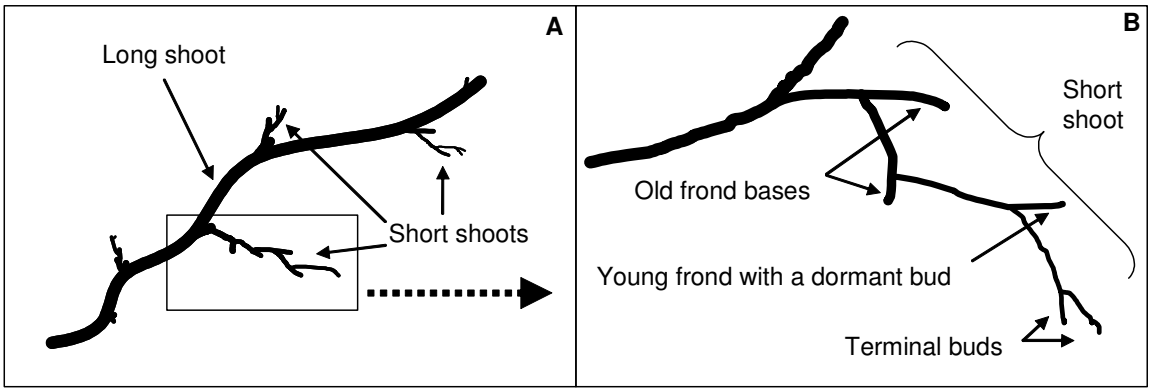

Fig. 1-3: Schematic structure of the rhizome system of bracken (A) and detail from A, showing the frond producing short shoot (B).

Due to the high growth rate of rhizomes [up to 112 cm per year (Watt, 1940)], bracken can quickly open up new areas substantiating its invasiveness (Conway, 1952). A high potential of regeneration from disintegrating rhizomes contributes to its invasive power. Clonal individuals regenerate even from short pieces of disintegrated rhizomes (Daniels, 1985). Thus, longevity for ages of a clone has been supposed (Oinonen, 1967) producing continuously new ramets (Watt, 1976). Contributing to its vigour are ***dormant buds*** which rest on the short shoots next to the basis of the petiole of each frond (Watt, 1940). Sprouting of these buds is apparently controlled by apical dominance from the living leaves and commences upon their natural senescence or premature destruction by adverse external factors like fire, frost, or pest control measures (Burge & Kirkwood, 1992). Bracken is a fire-tolerant species. Its buried rhizomes can rapidly produce new ***fronds*** after burning and, thus, outcompete other components of the vegetation (Flechter & Kirkwood, 1979). On heavily infested areas, bracken fronds can produce a closed canopy shortly after a fire (Roos *et al*., 2010) which by shading suppresses smaller plants (Burge & Kirkwood, 1992; Hartig & Beck, 2003). Allelopathic compounds of the fronds, largely

phenolic acids (Burge & Kirkwood, 1992; Alonso-Amelot *et al*., 2004), leaching from living and dead fronds are also considered as an important factor for the dominance of the fern (Gliessman, 1976). Pteridophytes in general produce a great variety of secondary metabolites, and this also holds true for bracken (Page, 1986). Toxicity of bracken fronds for mammals, causing anaemia and multiple haemorrhages, bright blindness, and tumors raises severe problems for livestock (Hannam, 1986). Secondary constituents have also been considered the reason for its poor palatability (Lawton, 1976) and pathogenic resistance (Cooper-Driver, 1976). Bracken ***spores*** have been reported as being carcinogenic, too (Evans & Galpin, 1990). From a fertile frond millions of extremely light spores can be released (Conway, 1957), which are dispersed by the wind and contribute to the fast spreading of the weed (Page, 1986). Long distance dispersal of spores from different origins might be one reason of the high genetic plasticity of *Pteridium*. Compatibility of gametes from the same gametophyte provides also the opportunity for genetic homozygosity in the case of an isolated progeny (Page, 1986).

High genetic plasticity allows an almost perfect adaptation to different habitats with a wide range of environmental conditions and makes bracken predominant in many parts all over the world. One of the main limiting factors of bracken growth is its sensitivity to frost, preventing its advance into arctic/subarctic and high alpine areas (Burge & Kirkwood, 1992). Another limitation is shade. Bracken is a light demanding species and, thus, can not invade forests with a closed canopy (Page, 1986). This plant is a specialist for disturbed areas and grows on different soils except compact waterlogged or saline soils, which hinder growth of the rhizome (Brown, 1986; Marrs & Watt, 2006).

Ecology of southern bracken. Whereas the literature on “northern bracken” and its ecology is substantial [e.g. bracken database from Crane (1990), available at: http://www.fs.fed.us/database/feis/plants/fern/pteaqu/all.html], information on the tropical bracken, especially on the neotropical species is very scarce. Only two studies exist from South America by Alonso-Amelot & Rodulfo-Baechler (1996) on bracken in Venezuela and by Quitete Portela *et al*. (2009) on Brazilian bracken.

Nothing is known about the local distribution of both neotropical bracken species in (South) Ecuador. In general, *P. caudatum* is more common in the lowlands while *P. arachnoideum* has its distribution optimum in the uplands climbing up to 3,000 m a.s.l.. In the Venezuelan Andes both species were found separately but also in mixed stands in the same type of habitat. There *P. arachnoideum* is taller than *P. caudatum* and the time period for leaf unfolding is almost twice as long (Alonso-Amelot & Rodulfo-Baechler, 1996). Any site preference of one of both species could not be observed. Respective of southern brackens ecology two aspects are of special interest: Its so-called fire tolerance and its competitive strength vis-à-vis crops, in particular pasture grasses. Tolerance of fire, which is a common agricultural tool of the local farmers for maintenance of the pastures, has been associated with the bracken rhizomes, which are deeply buried in the soil and, thus, are protected from damage by heat. Since these rhizomes are also the storage organs for organic reserves like carbohydrates, resprouting of new fronds from the rhizomes after a fire is possible already a few days after burning of the standing leaves. Surprisingly, the heat tolerance of bracken has not been studied in detail and only one publication by Flinn & Pringle (1983) reports on an experimental analysis of the heat tolerance of several rhizomatous species including northern bracken. In that experiment, bracken rhizomes proved not heat tolerant. However, in that study, rhizomes have not been separated in short and long shoots, and an assessment of a potential different heat tolerance of both types of shoots is not possible. Nothing is known about the heat tolerance of tropical bracken. Therefore, a comprehensive study was performed with bracken rhizomes from the study site in South Ecuador (Chapter 5).

When invading pastures, bracken has to compete with the present pasture grasses. In Brazil this is *Panicum maximum* JACQUIN (Quitete Portela *et al.*, 2009), while it is *Setaria sphacelata* (SCHUMACH.) STAPF & C.E. HUBB. in Ecuador (Hartig & Beck, 2003; Beck *et al.*, 2008a). Both grass species have a high capacity of biomass production and, perhaps, without grazing and periodically burning would be at least of similar competitive strength as bracken. Whereas burning damages both, the grasses and the fern, grazing only weakens the grass and in the long run, bracken takes over and finally outcompetes the grass (Hartig & Beck, 2003).

Ongoing bracken control measures concomitant with moderate grazing could stabilize the pastures, keeping bracken at a low level of infestation. However, no experiments have been reported so far on the control of southern bracken, although the demand is evident [40% infested pastures in the research area (Göttlicher *et al.*, 2009)].

Bracken control measures

One fifth of the bracken literature deals with control strategies, in particular on the northern hemisphere, demonstrating its economic importance as a weed. Due to the poor accessibility of the long shoots, pest control has proven extremely difficult, requiring continuous treatment over many years (Marrs *et al.*, 1998). Moderately successful control measures are permanent cutting of the fronds or treatment with specific herbicides, as well as combinations of both treatments (Pakeman *et al.*, 2000). Since cutting of the fronds also results in a release of the resting buds on the rhizomes from apical dominance (Burge & Kirkwood, 1992), at least two cuts per year have been recommended (Marrs *et al.*, 1998; Stewart *et al.*, 2008). Other suggested mechanical treatments are rolling/crushing and ploughing (Pakeman *et al.*, 2002). Many herbicides have been examined for control of bracken. Asulam, a systemic and to some extent selective herbicide, which in several countries has been registered for helicopter spraying, and the total herbicide glyphosate are commonly used (Burge & Kirkwood, 1992). Both achieve a reduction of frond density of more than 95% in the year after the treatment (Williams & Foley, 1975; Veerasekaran *et al.*, 1977; 1978), but complete eradication of all rhizomes is not possible. Irrespective of the applied control measure, follow-up treatments are necessary to keep infestation by bracken at a low level (Pakeman *et al.*, 2002).

Since bracken generates in many places more or less monotonous fern stands of large dimensions, another promising approach to combat bracken is biocontrol (Burge & Kirkwood, 1992). Two South African moth species, *Conservula conisigna* and *Panotima sp.* (Lawton *et al.*, 1988), and pathogenic fungi, like *Ascochyta pteridis, Phoma aquilina,* and *Stagonospora sp.* (Petrini *et al.*, 1992), were tested as

candidates for biological control. But up to now, none of these has been developed to a level of large-scale application (Fowler, 1993; Green, 2003).

While enormous research-directed efforts have been undertaken to control bracken in countries of the northern hemisphere (e.g. Marrs *et al.*, 1998; Pakeman *et al.*, 2000), reports on control of the even more aggressive tropical bracken with its all-year growth season are still lacking. Alarming invasion rates, for example from Mexico, where in the Southern Yucatán Peninsular Region the bracken infestation quadrupled between 1985 and 2001 (Schneider, 2004), show impressively that effective control measures are required to stop the loss of agricultural areas in the tropics. On that background a systematic study has been performed in the research area to sustainably control growth of bracken and regenerate pastures, which have been abandoned because of prevalence of bracken (Chapter 6).

THE STUDY SITE IN SOUTHERN ECUADOR

General facts

Ecuador, the smallest of the Andean countries with a territory of 256.370 km^2, is located at the west coast of South America between the 2^{nd} degree of latitude north and the 6^{th} degree south, and the 75^{th} and 81^{st} degrees of longitude west (Fig. 1-4A). The Republic of Ecuador has a population of about 14.5 millions inhabitants (CIA, 2009). The mainland can be differentiated into three regions: The coast (*costa*, ≤ 1000 m a.s.l.), the Andean highland (*sierra*, 1000 - 6400 m a.s.l.), and the Amazon lowland (*oriente*, < 1000 m a.s.l.). The Ecuadorian Andes consist of the western range (Cordillera occidental) and the eastern range (Cordillera oriental) and the innerandean highland basin. In spite of Ecuadors small territory, it belongs to one of the five hottest biodiversity hotspots of the world (Meyers *et al.*, 2000) but, on the other hand, has the highest deforestation rate of South America with an annual loss of 1.7% (between 2000 and 2005; FAO, 2009). This conflicting situation is quite obvious also in the particular research area in South Ecuador.

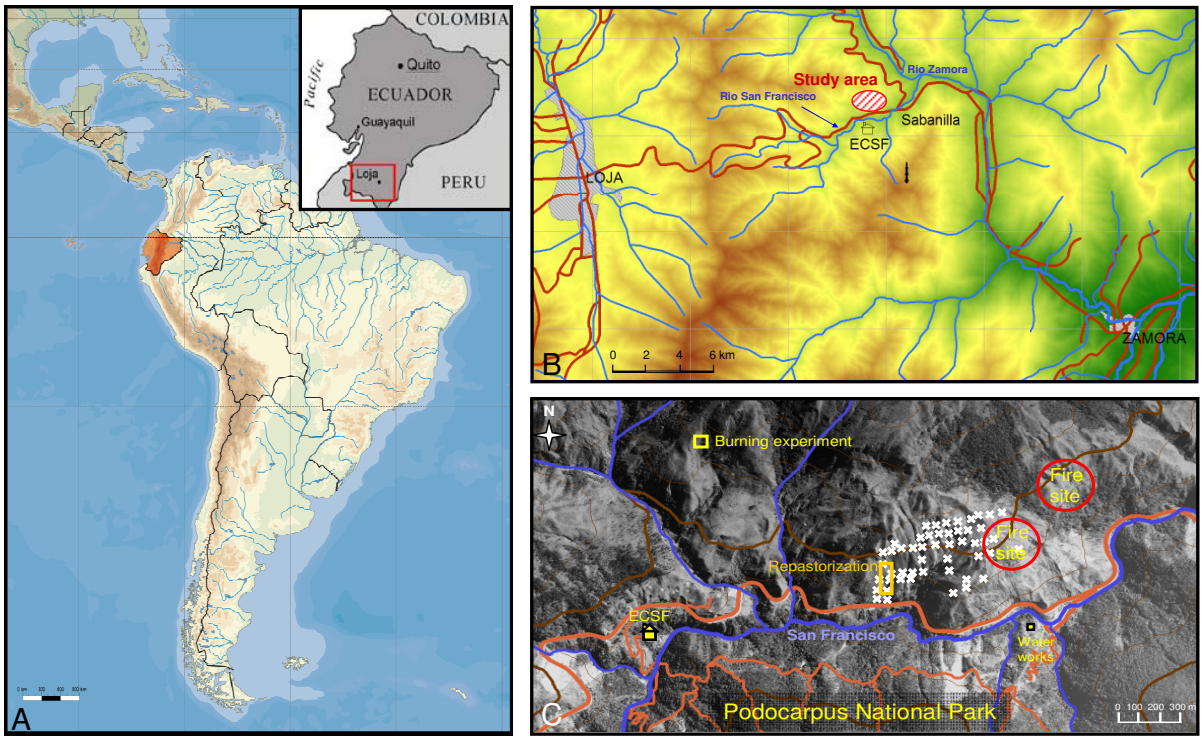

Fig. 1-4: Topography of the research site A) Ecuador (from Luizzo, 2008) and the location of the research area (from Beck *et al.*, 2008b), B) the research area in detail with the field station Estación Científica San Franscisco (ECSF) at 1,860 m a.s.l., C) aerial image with marked plot sites. White crosses: sampling sites of the population study (Chapter 3), red circles: observation sites of bracken regrowth after fires (Chapter 5), yellow square: experimental fire site (Chapter 5), orange rectangle: weed control and re-pasturisation site (Chapter 6).

The study area is located in the deeply incised Rio San Francisco valley in the eastern range of the Andes in South Ecuador and belongs to the province Zamora-Chinchipe (Fig. 1-4B and C, Beck *et al.*, 2008b). The orographically right site of the valley, the so called “Reserva Biológica San Francisco” (RBSF), which is part of the Podocarpus National Park (PNP), is covered by mostly undisturbed tropical mountain rain forest. At the opposite side, the forest has been widely cleared by slash and burn to gain pasture land. Because of the bracken problem, a substantial portion of the pastures has already been abandoned. The particular research area has a perhumid climate with an annual average temperature of 15.5°C and mean annual precipitation of 2050 mm (Bendix *et al.*, 2008). Parent rock material consists of Paleozoic slates, meta-siltstones, meta-sandstones, phyllites and quartzites. The catena of soil types

shows a high degree of heterogeneity (Schrumpf *et al.*, 2001). Cambisols, cambic Umbrisols and also Podzols of silt loam texture prevail (F. Haubrich, personal communication).

Since 1997, the research station ECSF is the logistic centre of comprehensive interdisciplinary ecosystem and biodiversity studies performed by an interdisciplinary German-Ecuadorian team with temporarily up to 30 research groups (www.bergregenwald.de and www.tropicalmountainforest.org). The projects started with an inventory of the abiotic and biotic components of the mountain rain forest investigating climate, hydrology, soils, flora, and fauna. Subsequently, these results were used to address ecosystem functioning. While the initial research projects concentrated on the natural forest, at present emphasis is also on the anthropogenic replacement ecosystems and their ecosystem services. With respect to the abandoned pastures and the invasion of bracken, experiments for reforestation and re-pasturisation of those areas have been started. In a complementing experiment the common practice of pasture burning is simulated. Chapter 6 of the work presented in this thesis focuses (in part) on the control of bracken and an attempt to re-pasturise the abandoned areas, using the common but introduced pasture grass *Setaria sphacelata*. Several other groups participate in these studies: Soil scientists from Dresden Technical University, plant ecophysiologists from the University of Osnabrück, hydrologists from the University of Giessen, mycologists from the University of Munich, and climatologists from the Universities of Erlangen and Marburg.

The present land use system of the local farmers

The territory of the northern buffer zone of the Podocarpus National Park is farmed by smallholders. Due to their different land use and management systems, a pattern of different types and intensities of anthropogenic interference could be spotted: Active and abandoned pastures, homegardens, maize or tree tomato fields, plantations of exotic tree species like pine (*Pinus patula*) or eucalypt (*Eucalyptus saligna*, *Eucalyptus globulus*), interspersed with remnants of the primary forest in the inaccessible ravines (Martínez Jerves, 2007).

The beginning of the non-sustainable land management is the extraction of high value timber (*Podocarpus oleifolius*, *Tabebuia chrysantha*, *Juglans neotropica*) from the pristine forest followed by burning of the degraded forests to completely clear the area. The resulting environmental conditions, bare soil enriched with nutrient from the ash, favours germination of bracken spores and growth of the gametophytes which mature in a short time period. Four to six weeks after the fire, vigorously sprouting bracken sporophytes were observed, forming patches of a lawn-like vegetation (Chapter 4). In addition, bracken rhizomes from neighbouring areas can enter the freshly cleared areas, intensifying the infestation. In the area, repeated burning of heavily infested pastures is the common practice for bracken control and for stimulation of the growth of pasture grass, but in the long run results in the dominance of the fern over the grass. Finally, after usually less than ten years, these pastures, when completely overgrown by the weed, are abandoned (Hartig & Beck, 2003; Beck *et al.*, 2008a). A land-cover classification of the area based on Landsat ETM+ data revealed that 40% of the pastures in the research area are already overgrown by bracken (Göttlicher *et al.*, 2009).

OBJECTIVES OF THE STUDY

The overall motivation of the study was to contribute to a better and sustainable management of the pastures in the humid part of the South Ecuadorian Andes, and thus to alleviate the pressure on the remaining natural forests. With regard to the scarce information about the biology of southern bracken in Ecuador, this study should contribute to a better knowledge of the species composition and the ecology of this weed with particular emphasis on its response to burning. Finally, an extensive experiment was performed over four years, testing mechanical, chemical and physical methods of bracken control with regard to a subsequent replanting of the common pasture grass *Setaria sphacelata*.

In addition to the *Introduction*, a *Synopsis* of the results and a *Summary*, this thesis consists of four chapters with the following content:

1. **Determination of bracken species in the research area and analysis of their genetic structure and population diversity** (*Chapter 3*)

2. **Short description of the present agricultural land use system and identification of bracken as a dominant pasture weed in southern Ecuador** (*Chapter 4*)

3. **General growth observation of tropical bracken and its responses to climate extremes** (*Chapter 5*)

4. **Investigation of tropical bracken's fire ecology on the basis of field observations and a laboratory experiment** (*Chapter 5*)

5. **Identification of potential control measures for tropical bracken** (*Chapter 6*)

6. **Analysis of the possibility of re-pasturisation of abandoned bracken infested areas** (*Chapter 6*)

REFERENCES

Alonso-Amelot ME, Rodulfo-Baechler S (1996) Comparative spatial distribution, size, biomass, and growth rate of two varieties of bracken fern (*Pteridium aquilinum* L. Kuhn) in an neotropical montane habitat. Vegetatio 125: 137-147.

Alonso-Amelot ME; Oliveros A; Calcagno-Pisarelli MP (2004) Phenolics and condensed tannins in relation to altitude in neotropical *Pteridium* spp. A field study in the Venezuelan Andes. Biochemical Systematics and Ecology 32: 969-981.

Ashcroft C, Sheffield E (1999) Rejection of *Pteridium aquilinum* subspecies *atlanticum* (C.N. Page). Botanical Journal of the Linnean Society 130, 157-170.

Beck E., Hartig K, Roos K (2008a) Forest clearing by slash and burn. In: Beck E, Bendix J, Kottke I, Makeschin F and Mosandl R (eds.) Gradients in a Tropical Mountain Ecosystem of Ecuador. Ecological Studies, Vol. 198. Springer, Berlin Heidelberg, pp 371-374.

Beck E, Makeschin F, Haubrich F et al. (2008b) The ecosystem (Reserva Biológica San Francisco). In: Beck E, Bendix J, Kottke I, Makeschin F and Mosandl R (eds.) Gradients in a Tropical Mountain Ecosystem of Ecuador. Ecological Studies, Vol. 198. Springer, Berlin Heidelberg, pp 1-13.

Bendix J, Rollenbeck R, Richter M, Fabian P, Emck P (2008) Climate. In: Beck E, Bendix J, Kottke I, Makeschin F and Mosandl R (eds.) Gradients in a Tropical Mountain Ecosystem of Ecuador. Ecological Studies, Vol. 198. Springer, Berlin Heidelberg, pp 63-73.

Brown R (1986) Bracken in the North York Moors: Its ecological and amenity implications in national parks. In: Smith, RT and Taylor, JA. Bracken: Ecology, Land Use and Control Technology. The Parthenon Publishing Group, Leeds Lancs, pp 453-458.

Burge M, Kirkwood R (1992) The control of bracken. Critical Reviews in Biotechnology 12: 299-333

Chen XQ, Wang JX, Tian HL, Zhang XC, Wen J, Zhou SL (2008) Development of microsatellite markers for the bracken fern, *Pteridium aquilinum*. Molecular Ecology Resources 8, 1491-1493.

CIA (2009) The world factbook. https://www.cia.gov/library/publications/the-world-factbook/index.html. Accessed February 2010.

Cobb B, Farnsworth E, Lowe C (2005) A field guide to ferns and their related families. The Peterson field guide series. The New England Wild Flower Society. Houghton Mifflin Co. Boston.

Conway E (1952) Bracken – the problem plant. Scottish Agriculture 31: 181-184.

Conway E (1957) Spore production in bracken (*Pteridium aquilinum* (L.) Kuhn). Journal of Ecology 45: 273-284.

Cooper-Driver G, Finch S, Swain T, Bernays E (1977) Seasonal variation in secondary plant compounds in relation to the palatability of *Pteridium aquilinum*. Biochemical Systematics and Ecology 5: 177-183.

Daniels R (1985) Studies in the growth of *Pteridium aquilinum* (L.) Kuhn (bracken) .1. Regeneration of rhizome segments. Weed Research 25: 381-388.

Der J, Thomson JA, Stratford J, Wolf P (2009) Global chloroplast phylogeny and biogeography of bracken (*Pteridium*; Dennstaedtiaceae). American Journal of Botany 96: 1041-1049.

Evans IA, Galpin O (1990) Bracken and leukemia. Lancet 335: 231.

FAO (2009) State of the World's Forests 2009. Food and Agricultural Organization of the United Nations, Rome.

Flechter WW, Kirkwood RC (1979) The bracken fern (*Pteridium aquilinum* L. (Kuhn)); its biology and control. In: Dyer AF (ed.): The Experimental Biology of Ferns. Academic Press, New York, pp 591-635.

Flinn M,Pringle J (1983) Heat tolerance of rhizomes of several understory species. Canandian Journal of Botany 61: 452-457.

Fowler S (1993) The potential for control of bracken in the UK using introduced herbivorous insects. Pesticide Science 37: 393-397.

Gliessman S (1976) Allelopathy in a broad spectrum of environments as illustrated by bracken. Botanical Journal of the Linnean Society 73: 95-104.

Green S (2003) A review of the potential for the use of bioherbicides to control forest weeds in the UK. Forestry 76: 285-298.

Göttlicher D, Obregón A, Homeier J, Rollenbeck RNT, Bendix J (2009) Land-cover classification in the Andes of southern Ecuador using landsat ETM+ data as a basis for SVAT modelling. International Journal of Remote Sensing 30: 1867-1886.

Hannam DAR (1986) Bracken poisoning in farm animals with special reference to the North York Moors. In: Smith, RT and Taylor, JA. Bracken: Ecology, Land Use and Control Technology. The Parthenon Publishing Group, Leeds Lancs, pp 133-138.

Harper, JL (1982) Population Biology of Plants. Academic Press, London, New York, San Francisco. p 729

Hartig K, Beck E (2003) The bracken fern (*Pteridium arachnoideum*) dilemma in the Andes of South Ecuador. Ecotropia 9, 3-13.

Holm L, Doll J, Holm E, Pancho J, Herberger J (1997) World weeds: Natural histories and distribution. Wiley, New York.

Korpelainen H (1995) Mating system and distribution of enzyme genetic variation in bracken (*Pteridium aquilinum*). Canadian Journal of Botany 73, 1611-1617.

Lawton J (1976) Structure of arthropod community on bracken. Botanical Journal of the Linnean Society 73: 187-216.

Lawton JH, Rashbrook VK, Compton SG (1988) Biocontrol of British Bracken: The potential of two moths from southern Africa. Annals of Applied Biology 112: 479-490.

Lellinger D (1985) A field manual of ferns and fern-allies of the United States and Canada. Smithsonian Institution Press. Washington, pp 209-212.

Luizzo DS (2008) South America location. http://de.wikipedia.org/wiki/Ecuador. Accessed February 2010.

Marrs R, Johnson S, Le Duc M (1998) Control of bracken and restoration of heathland. VI. The response of bracken fronds to 18 years of continued bracken control or 6 years of control followed by recovery. Journal of Applied Ecology 35: 479-490.

Marrs R, Watt A (2006) Biological flora of the British Isles: *Pteridium aquilinum* (l.) Kuhn. Journal of Ecology 94: 1272-1321.

Martínez Jerves JA (2007) Sekundäre Bergregenwälder in Südecuador: Der Einfluss der Art der Störung auf das Spektrum der Pflanzenarten und die Waldstruktur, eine vegetationskundliche Analyse. University of Bayreuth, PhD thesis.

Myers N, Mittermeier R, Mittermaier C, da Fonseca G, Kent J (2000) Biodiversity hotspots for conservation priorities. Nature 403: 853-8.

Oinonen E (1967) The correlation between the size of Finnish Bracken (*Pteridium aquilinum* (L.) Kuhn) clones and certain periods of site history. Acta Forest Fenn 83: 47-97.

Page C (1976) Taxonomy and phytogeography of bracken - a review. Botanical Journal of the Linnean Society 73: 1-34.

Page C (1986) The strategies of bracken as a permanent ecological opportunist. In: Smith, RT and Taylor, JA. Bracken: Ecology, Land Use and Control Technology. The Parthenon Publishing Group, Leeds Lancs, pp. 173-181.

Page C (1989) Three subspecies of bracken, *Pteridium aquilinum* (L.) Kuhn, in Britain. Watsonia 17: 429-434.

Page C (1994) Structural variation in Western European Bracken: An updated taxonomic perspective. In: Smith, RT: Bracken Conference. International Bracken Group, Aberystwyth , pp 13-15.

Page C (2002) Ecological strategies in fern evolution: a neopteridological overview. Review of Palaeobotany and Palynology 119: 1-33.

Page C, Mill R (1995a) Scottish bracken (*Pteridium*): New taxa and a new combination. Botanical Journal of Scotland 47: 139-140.

Page C, Mill R (1995b) The taxa of Scottish Bracken in a European perspective. Botanical Journal of Scotland 47: 229-247.

Pakeman R, Le Duc M, Marrs R (2000) Bracken distribution in Great Britain: Strategies for its control and the sustainable management of marginal land. Annals of Botany 85: 37-46.

Pakeman R, Le Duc M, Marrs R (2002) Bracken control, vegetation restoration and land management. Food & Rural Affairs, Rural Development Service Technical Advice Note 1.

Pakeman R, Marrs R, Jacob P (1994) A model of bracken (*Pteridium aquilinum*) growth and the effects of control strategies and changing climate. Journal of Applied Ecology 31: 145-154.

Petrini O, Fisher P, Petrini L (1992) Fungal endophytes of bracken (*Pteridium aquilinum*), with some reflections on their use in biological control. Sydowia 44: 282-293.

Quitete Portela RC, Silva Matos DM, Pugliese de Siqueira L. et al. (2009) Variation in aboveground biomass and necromass of two invasive species in the Atlantic rainforest, Southeast Brazil. Acta Botanica Brasilica 23: 571-577.

Roos K, Rollenbeck R, Peters T, Bendix J, Beck E (2010) Growth of tropical bracken (*Pteridium arachnoideum)*: Response to weather variations and burning. Invasive Plant Science and Management. DOI: 10.1614/IPSM-D-09-00031.1.

Schneider LC (2004) Bracken fern invasion in Southern Yucatán: A case for land-change science. The Geographical Review 94: 229-241.

Schrumpf M, Guggenberger G, Valarezo C, Zech W (2001) Tropical montane rain forest soils. Development and nutrient status along an altitudinal gradient in the South Ecuadorian Andes. Die Erde 132: 43-59.

Sheffield E, Wolf PG, Rumsey FJ, Robson DJ, Ranker TA, Challinor SM (1993) Spatial distribution and reproductive behavior of a triploid bracken (*Pteridium aquilinum*) clone in Britain. Annals of Botany 72: 231-237.

Speer WD (1999) A systematic assessment of British and North American *Pteridium* using cpDNA gene sequences. Taylor JA, Smith RT (eds.) *Bracken Fern: Toxicity, Biology and Control*. Aberystwyth: International Bracken Group, pp. 37-42.

Speer WD (2008) Phylogenetic and biogeographic relationships among North American and Hawaiian *Pteridium aquilinum* (L.) Kuhn (Dennstaedtiaceae) based on chloroplast *rps4* and *rps4-trnS* intergenic spacer sequences. American Fern Journal 98, 179-193.

Speer WD, Werth CR, Hilu KW (1999) Relationships between two infraspecific taxa of *Pteridium aquilinum* (Dennstaedtiaceae). II. Isozyme evidence. Systematic Botany 23, 313-325.

Stewart G, Cox E, Le Duc M, Pakeman R, Pullin A , Marrs R (2008) Control of *Pteridium aquilinum*: Meta-analysis of a multi-site study in the UK. Annals of Botany 101: 957-970.

Thomson J (2000) Morphological and genomic diversity in the genus *Pteridium* (Dennstaedtiaceae). Annals of Botany 85: 77-99.

Thomson J, Alonso-Amelot M (2002) Clarification of the taxonomic status and relationships of *Pteridium caudatum* (Dennstaedtiaceae) in Central and South America. Botanical Journal of the Linnean Society 140: 237-248.

Tryon RM (1941) A revision of the genus *Pteridium*. Rhodora 43: 1-31, 37-67.

Tryon R, Tryon A (1982) Ferns and allied plants - with special reference in tropical America. Springer Verlag.

Veerasekaran P, Kirkwood R, Fletcher W (1977) Studies on mode of action of asulam in bracken (*Pteridium aquilinum* L. Kuhn).1. Absorption and translocation of [Asulam-c-14]. Weed Research 17: 33-39.

Veerasekaran P, Kirkwood R, Fletcher W (1978) Studies on mode of action of asulam in bracken (*Pteridium aquilinum* L. Kuhn).3. Longterm control of field bracken. Weed Research 18: 315-319.

Watt A (1940) Contributions to the ecology of bracken (*Pteridium aquilinum*).I. The rhizome. New Phytol. 39: 401-422.

Watt A (1976) The ecological status of bracken. Botanical Journal of the Linnean Society 73: 217-239.

Webster B, Steeves T (1958) Morphogenesis in *Pteridium aquilinum* (L.) Kuhn. General morphology and growth habit. Phytomorphology 8: 30-41.

Williams G, Foley A (1975) Effect of herbicides on bracken rhizome survival. Annals of Applied Biology 79: 109-111.

Wolf PG, Haufler CH, Sheffield E (1988) Electrophoretic variation and mating system of the clonal weed *Pteridium aquilinum* (L. Kuhn) (bracken). Evolution 42, 1350-1355.

Wolf PG, Sheffield E, Haufler CH (1991) Estimates of gene flow, genetic substructure and population heterogeneity in bracken (*Pteridium aquilinum*). Biological Journal of the Linnean Society 42: 407-423.

CHAPTER 2

Synopsis

SPECIES COMPOSITION OF SOUTHERN BRACKEN IN THE RESEARCH AREA (CHAPTER 3)

The first comprehensive monograph of the genus *Pteridium* (bracken) was based on geographical distribution patterns, and morphological and anatomical characters (Tryon, 1941). Although these are still useful characters, their relevance is not sufficient for a modern taxonomic analysis of the worldwide distributed bracken fern. Systematics of the complex "*Pteridium aquilinum* s.l." is in particular complicated by a phenotypic plasticity and a high potential for hybridization, allopolyploidy and apomixis (Tryon, 1941; Daniels, 1985; Sheffield *et al.*, 1993; Thomson, 2000). It is self-evident that continental separation due to prevention of gene exchange results in independent radiation and speciation and sooner or later in distinct races, subspecies or species. Considering the geological age of the fern group Dennstaedtiaceae (Triassic) and of the genus Pteridium (from the Eocene) the worldwide relative uniformity of bracken is surprising. However, in view of the predominantly vegetative propagation of this rhizomatous fern, sexual propagation could be less frequent and non-significant for the success of the various species of this genus. Apart from different concepts of the taxonomic ranking of individual bracken forms, there is general agreement that the genus Pteridium consists of a so-called "northern", Laurasian and partly African clade and a "southern", mostly tropic clade comprising bracken in the Neotropics, Oceania, and part of Africa (Der *et al.*, 2009). This concept is mainly based on the application of molecular markers (Speer *et al.*, 1999; Thomson, 2000; Der *et al.*, 2009). Such markers are also useful tools on a lower taxonomic level, e.g. the identification of species and the genetic clarification of intermediate morphotypes and hybrids. As with all kinds of markers, some uncertainty remains, irrespective of the method. In bracken taxonomy and species identification allozyme analysis (Wolf *et al.*, 1991; Speer *et al.*, 1999), DNA fingerprinting by arbitrarily-primed PCR (Thomson, 2000, 2008), and sequencing of the chloroplast *rps4* and *rps4-trnS* intergenic spacer region (Speer, 2008; Der *et al.*, 2009) has been used. Recently, microsatellite analysis has been added to this toolbox, in particular for identification and differentiation of bracken individuals. Microsatellite analysis represents a promising molecular method due to the co-dominant inheritance of microsatellites and because of their high level of

polymorphism. Up to the present, only a short communication on the development of bracken microsatellites has been published (Chen *et al.*, 2008), and thus their applicability still needs proof.

In the study presented in Chapter 3, the already approved molecular methods of allozyme analysis and sequencing of *rps4* and *rps4-trnS* region were combined with microsatellite analysis in addition to the morphological characters of the bracken leaves. This combined approach was used to assess bracken diversity and species composition of bracken in the study area of the Rio San Francisco valley. In this study, 50 bracken samples were collected from a heavily bracken-infested area of 19.25 ha between 1,800 and 2,100 m a.s.l.. Due to the applied methods, 29 samples were identified as the diploid *Pteridium arachnoideum*, 20 samples as the tetraploid *Pteridium caudatum*, and one as a potential hybrid. Using UPGMA-based tree clustering of D_{TL} distances as well as Bayesian clustering, similar relationships between the individuals of *P. arachnoideum* on the one hand and *P. caudatum* on the other could be shown. Comparison of the observed and the expected degrees of heterozygosity indicates a higher gene flow and population stability for *P. arachnoideum* than for *P. caudatum* which at the study site reaches its upper altitudinal limit. Combining all methods of analysis, only two samples (*P. arachnoideum*) were identical, indicating either two samples from one individual or two ramets of the same genet. Because these samples were collected in a distance of 50 meters the extension of one individual or genet of the tropical species appears considerably smaller than that reported for the northern bracken. This is very interesting, since bracken is a clonal species and genet extensions of representatives of the northern bracken investigated with allozyme analyses were estimated as 390 meters up to over 1,000 meters (Sheffield *et al.*, 1989; Parks & Werth, 1993). In the humid tropics, where growth is not interrupted by a winter break, even larger areas of one bracken genet could have been expected. Ecological preferences of both *Pteridium* species could not be recognized as both were found randomly distributed growing side by side on the study site.

Since in field experiments separation of the two species which are growing in mixed stands is not possible, the reactions of the species could not be recorded separately. Likewise, in the heat-pulse experiment, the species could not be treated individually since there is no character for differentiation between the rhizomes of P. arachnoideum *and* P. caudatum*. Therefore, the other chapters refer to tropical bracken as a mixture of both species.*

THE USE OF FIRE FOR DEFORESTATION (CHAPTER 4)

Chapter 4 reflects on the present agricultural land use system in southern Ecuador. As elsewhere in the tropics, farmers in the Andes of South Ecuador make extensive use of fire to convert primary forest into farming land and to maintain their pastures. The remnants of previous burnings – charred trunks and large branches – are frequently left where they had fallen, because of the enormous efforts to remove them from the steep and often remote areas. Many areas have thus to be burned repeatedly until the spaces between the remaining logs are wide enough to plant beans or maize, or the pasture grasses *Setaria sphacelata* and *Melinis minutiflora*. A few months after fire bracken (*Pteridium* spp.) was observed, sprouting vigorously on both the burnt and the heat-killed areas, while it was absent in the intact primary forest. Bracken and crops develop simultaneously after burning. Sooner or later the crops are usually replaced by tillering pasture grasses, in particular *Setaria sphacelata*, while the bracken fronds protrude mainly from spaces between the tussocks. At the beginning, *Setaria* grows faster than bracken and forms homogeneous pastures. However, since only the very young leaf blades and the tips of mature leaves are eaten by cattle, the carrying capacity of these pastures is low. Bracken is not eaten due to its toxicity (Evans & Galpin, 1990; Hannam, 1986; Fenwick, 1989). For pasture rejuvenation and weed killing, farmers set fire whenever the weather permits. Repeated burning of the pasture land together with grazing weakens the competitive strength of the pasture grasses, but increases the competitive strength of the extremely aggressive and – because of its subterranean rhizome system - fire-tolerant bracken. Pastures are finally abandoned when bracken becomes completely dominant. On steep slopes wind-dispersed seeds of several

bushes like *Baccharis latifolia* and *Ageratina dendroides* from the Asteraceae or *Monochaetum lineatum* and *Tibouchina laxa* from the Melastomataceae germinate in the shade of the bracken leaves. A long-lasting successional vegetation composed of dense patches of bracken interspersed with individual bushes develops.

Observing the land use management of the local farmers, the so-called newcomers or colonos, clearly revealed that the present land use system in southern Ecuador is not sustainable. Southern (tropical) bracken was recognized as an extremely aggressive weed which readily invades the steep slopes of the humid Andean valleys in Ecuador and presumably similar sites in other Andean countries. Only on the small flattenings pasture grasses and herbs can compete with bracken whose rhizomes can not grow in compacted and waterlogged soil. After several years of recurrent burning of the pastures bracken takes completely over and due to the lack of manpower and financial means for weed control the areas are abandoned and clearing of the remaining natural mountain forest continues. Whereas bracken on the northern hemisphere has been extensively studied, only a few publications report on the ecology of tropical bracken, which, however, because of an all-year vegetation period grows taller and is much more aggressive than its northern relatives. One major goal of the present study was therefore to add to the knowledge on the biology and ecology of southern bracken, and in particular to those traits which make it so aggressive (Chapter 5). On the basis of such knowledge a broad bracken control experiment with the perspective of re-pasturisation of the abandoned areas was conducted (Chapter 6).

BRACKEN GROWTH AND ITS REACTION TO FIRE (CHAPTER 5)

Undisturbed growth and response to particular weather situations

While the lifetime of the fronds of the northern bracken is limited to the frost-free months of the year, that of the tropical bracken is not subjected to such seasonal constraints. Its lifetime may be regulated by endogenous factors resulting in “natural” senescence and death. Therefore, the developmental phases of the fronds of

bracken were monitored. At a densely covered bracken slope within an area of 3.5 ha, plots were randomly established and observed from November 2005 until May 2008. Bracken fronds were tagged upon emergence and their developmental stages were recorded monthly. The life-time of the individual fronds varied between four and ten months with a median of eight months which is definitely longer than the temperature-limited life-span of leaves of northern bracken. Unfolding took less than one month, the length of the mature state was between three and seven months, and the phase of senescence lasted up to two months from the first incidence of yellowing until browning and drying-up of the entire frond. An almost perfect one to one ratio of emerging and dying leaves was found, which suggested limitation of frond density by nutrient shortage. In this case the development of new leaves requires nutrients remobilized from senescing leaves. Bracken generally prefers soils with a medium to high nutrient content (Waring & Major 1964; Carlisle *et al.*, 1967; MacLean *et al.*, 1977; Ader, 1990), but especially the topsoils of the study site are poor in macronutrients (Hamer *et al.*, 2009).

On all plots the balanced bracken frond density showed only short-term deviations from the one to one ratio. These could be correlated with weather extremes. Episodic spells of sunny days with high solar radiation foster frond emergence, while those of dry days (which in this region means a humidity < 70%) match frond mortality. For the northern bracken, Roberts *et al.* (1980) showed that above average transpirational losses during exceptionally warm and dry periods resulted in enhanced frond mortality and a substantial reduction of bracken leaf area index indicating the sensitivity of bracken to a high vapour pressure deficit. Obviously the tropical bracken is no exception to this trait.

Response of bracken to fire/heat

Fire, used as an agricultural tool for rejuvenation of pastures in southern Ecuador, results in a more vital establishment of bracken (Chapter 4) and, thus, is one possible reason of the high abundance of this weed in the research area. There are numerous reports (e.g. Agee & Huff, 1987; Ingram, 1931; Sharik et al., 1989) that fire, through killing the standing fronds, stimulates growth of bracken. However, in an experiment

by Flinn and Pringle (1983), rhizomes of northern bracken proved poorly heat resistant. To analyse whether the same holds also for the tropical bracken, field observations combined with heating experiments in the laboratory were conducted (second part of Chapter 5).

Field observations

In October 2004 and December 2005 two bushfires devastated major parts of the study area, where bracken was already dominating the vegetation. On randomly established plots in an area of 7.4 ha, regrowth of bracken was recorded for 18 months after the first fire, and on an area of 3.7 ha for 12 months after the second. A burst of leaf production was recorded two months after the fire and, depending on the original density of the bracken rhizomes, formed a more or less closed bracken canopy. Maximum bracken frond density was observed between five and seven months of regeneration. In addition to the stimulation of frond emergence, burning of the vegetation had a synchronizing effect on the development of bracken leaves. Mass dying of fronds concomitantly with the emergence of new ones was observed when its maximum life-time was reached. Thereafter a slow decrease of bracken frond coverage by 23% on average was observed until a stable level was reached. Infraspecific competition resulting in self-thinning appears to be the cause for this reduction. Northern bracken is known as a light demanding, shade intolerant pioneer species (Ingram, 1931; Page, 1986; Stewart, 1988) and the southern bracken apparently is no exception to this. Overlapping of the fronds resulting in a steep gradient of light intensity in the canopy layer may cause premature senescence of succumbing fronds.

Records of individually tagged leaves showed a statistical significant increase of the life-time of the fronds (nine months) produced after the fire as compared to that of the leaves in the undisturbed plots (eight months). The increased life-span by one month is in line with the idea that low nutrient availability restricts the life-time of fronds in the undisturbed plots. In a perhumid climate nutrients are quickly allocated after a fire from the ash to the topsoil, thus alleviating the nutrient shortage of the

plant for a while. An improved vitality of post-fire sprouts has also been reported for northern bracken (Page, 1982).

Heat pulse experiment

From a pilot burning experiment it was known that only a slight increase of the soil temperature follows the usually low-intensity grass fires. Already in two centimetres depth the moist soil heated up to not more than 40°C. The highest density of bracken rhizomes in the pastures of the study area was found between two and nine centimetres (Chapter 4). To investigate the physiological effects of this moderate heat shock on tropical bracken, excavated rhizomes were subjected to heat pulses of different time spans at various elevated temperatures. Apart from the comparative study of the heat tolerance of several rhizomatous plant species by Flinn and Pringle (1983), no data of the effects of experimental heat pulses on bracken rhizomes have been reported so far. These authors immersed rhizome pieces of various lengths for five minutes in a water bath which had been preheated to temperatures between 45 and 60°C. In nature, rhizomes of the tropical bracken are commonly embedded in moist soil and, therefore, in the work presented here heat pulses were applied in two ways: With and without original soil. By their structure, bracken rhizomes can be differentiated into the frond producing short or lateral shoots and the nutrients storing and rapidly growing long shoots. In the heating experiment described below, short and long shoots were treated separately. In contrast to our pilot burning experiment, De Bano (1977) reported soil temperatures of up to 80°C at five centimetres soil depth during a bushfire. Therefore, rhizomes were subjected either to a short heat shock of temperatures between 40 and 80°C in a water bath or heated for ten minutes in soil. After the treatments, the rhizomes were planted in their original soil and kept under natural conditions except controlled irrigation. After two months of cultivation, the physiological condition, increment, and frond production was recorded. The short shoots survived temperatures up to 80°C and, thus, were significantly more heat resistant than the long shoots, which hardly survived 60°C. As heat tolerance of the short rhizomes was remarkably high, they have a good chance for surviving the grass and bush fires lit by the farmers.

After a fire, bracken rapidly invades the cleared areas, giving rise to the hypothesis that the heat pulse stimulates extension growth of the rhizomes, especially of the long shoots. However, the contrary was observed with long shoots whose extension growth was even reduced by the heat treatment. In contrast, extension growth of the short shoots was slightly, but significantly promoted by the heat pulse in soil at 40 and 55°C, and at 50°C in the water bath. Whether this results from a direct effect of the heat pulse on the growth of the rhizome or reflects a positive effect of the heat pulse on the bracken mycorrhiza in the simultaneously heated soil remains an open question. *Pteridium aquilinum* is known to develop an endotrophic mycorrhiza (Conway & Arbuthnott, 1949; Tempel, 1981) and this probably holds also for southern bracken in the research area (K. Hartig and I. Kottke, personal communication).

In line with the field observations after a fire, a significant stimulation of frond production from short shoots was observed after a heat pulse between temperatures of 40/45°C and 55°C. The positive effect of the heat pulse was more pronounced when the rhizomes were heated in soil as compared to the wet heat pulse in the water bath. It is known that production of a leaf could inhibit sprouting of neighbouring buds by apical dominance (Burge & Kirkwood, 1992).

To examine whether the initial burst of leaf emergence after a fire/heating could also be explained by an alleviation of apical dominance after killing the fronds, regrowth of bracken on three moderately infested plots was compared after controlled burning and after cutting of the fronds. Cutting resulted in a recovery of the initial density after three months, whereas twice as much fronds emerged after burning. Thus, additional stimulation of buds by the heat pulse was clearly shown.

The rhizome system is the most important feature of bracken for surviving adverse environmental conditions (Burge & Kirkwood, 1992). It could be shown in Chapter 5 that the invasive power of tropical bracken obviously rest primarily with the more heat tolerant short shoots, which respond to a moderate heat pulse with enhanced extension growth and frond production. The long shoots, which due to their location

in greater soil depth are usually not affected by the heat of a bush- or grassfire, contribute to fast encroachment of new areas by their own extension growth and by providing nutrients to the lateral shoots; and with regard to control measures, it is the long shoot that warrants survival. Due to the poor accessibility of the long shoots, pest control has proven extremely difficult requiring continuous treatment over many years to deplete the long shoots from reserves (Marrs et al., 1998). Another reason for the difficulties in bracken control is the high potential for regeneration from even small fragments of the rhizome system (Daniels, 1985). As a consequence irrespective of the applied control measure, follow-up treatments are necessary for keeping infestation by bracken at a low level (Pakeman et al., 2002). In contrast to the ample literature on the control of northern bracken (see e.g. Stewart et al., 2008, Cox et al., 2008), respective reports on the even more aggressive neotropical bracken are completely lacking. For the locally used herbicides, the instructions of the producers were the only available information. The results presented in Chapter 6 are thus the first reports on control of bracken in the neotropics.

CONTROL OF TROPICAL BRACKEN (CHAPTER 6)

Long-term efforts were necessary for a decent control of northern bracken irrespective of the method (Pakeman *et al.*, 2000). Cutting of the fronds twice a year, application of specific herbicides, or combinations of both were the most successful treatments (Pakeman *et al.*, 2000). Many herbicides have been tested for bracken control (Burge & Kirkwood, 1992) and Picloram (a systemic broadband herbicide) and Metsulfuron-methyl (a systemic sulfonylurea herbicide) have proven effective (Farnworth & Davies, 1974; West & Richardson, 1985). But today, asulam [a systemic carbamate herbicide that is selective for weeds with a strong subterranean storage organ (Pakeman *et al.*, 1998; Snow & Marrs, 1997)] as well as the total herbicide glyphosate (“Roundup”) are more commonly applied in control of northern bracken. With both a temporary reduction of frond density of more than 95% in the year after the treatment was achieved (Williams & Foley, 1975; Veerasekaran *et al.*, 1977, 1978). For the neotropical bracken control experiments have not yet been described. Considering the necessity of practical orientation affordable methods and

locally available herbicides were preferred but also treatments which refer to the ecological requirements of bracken were tested.

For the bracken control experiment in the San Francisco valley, an area with a bracken cover of about 80% was chosen. The following 13 control measures were applied over a time period of 23 months: (1) mechanical control by cutting of the fronds; (2) chemical control with herbicides: asulam, glyphosate, a herbicide combination of picloram and metsulfuron methyl (for convenience the term “PM” is used) and pinolene as a transpiration blocker; (3) physical control by covering the plot with transparent plastic foil (exclusion of rain) or black plastic foil (exclusion of rain and light). Treatments were applied at least six times in intervals of four to six months. Most effective measures were the application of the herbicide combination PM and the mechanical treatment by cutting the fronds with ca. 65% reduction of bracken biomass after the treatment. Also the treatment with black foil and two combined treatments (PM + cutting) and (black foil + cutting) were fairly effective with ca. 40% reduction in comparison with the controls. To put into practice, it is important to know how many treatments are required for a clear control effect. To address that problem, resprouting of bracken in the course of the post-treatment phases was monitored monthly. For the five most effective treatments the minimum number of repetitive applications were between two (PM + cutting) and four (cutting, PM, black foil, black foil + cutting).

In the long run, a stable weed vegetation develops on abandoned pastures (Hartig & Beck 2003, Beck *et al*., 2008). Aiming at reconverting these into pastures, also the shrubs must be removed. The herbicide PM was most effective in that respect and more than 90% of the shrubs died during the treatment period of 23 months. Since PM was also effective in bracken control, this locally available herbicide is the straight choice for application in the research area, best in combination with regular cutting of the bracken fronds.

Nevertheless complete eradication of bracken is practically impossible even upon long-term application of control measures. Therefore, an extended control strategy is

necessary for re-pasturisation *of the abandoned areas. In principle such strategy is self-evident: Planting a pasture grass with a comparable or even higher competitive strength as bracken, after at least transitorily removal of the fern from the area. The introduced and commonly planted pasture grass* Setaria sphacelata *is everywhere available and therefore was the first choice for a* re-pasturisation *experiment. The second part of Chapter 6 shows the results of this approach.*

RE-PASTURISATION WITH A COMMON PASTURE GRASS (CHAPTER 6)

After the final bracken control treatment, the plots were left fallow for two months before *Setaria sphacelata* was planted in the customary way. Apart from negligible losses upon transplantation all plantlets survived and after three months the grass was vigorously growing. Concomitantly with the growth of *S. sphacelata*, resprouting of bracken was observed but at reduced rates. Initially differences in growth of bracken and *S. sphacelata* were seen, which could have resulted from aftermath effects of the bracken control treatments, but 1.5 years after replanting *S. sphacelata*, these differences had equalized and all plots were clearly dominated by the grass. Alleviation of the respective differences evidences the extraordinary competitive power of *S. sphacelata*. Thus, this grass turned out as a suitable candidate for re-pasturisation of abandoned pastures in Ecuador. Interestingly, the method of bracken control did not play a significant part in the final success of re-pasturisation. Although the fern could not overtop the grass during the more than two years period of observation, some additional weeding of the pastures is advisable. For this purpose and for rejuvenation of the tall grass bunches local farmers commonly burn the pastures from time to time. As described in Chapters 4 and 5, this measure benefits the bracken, and in the long run results in abandonment of the pastures. Since application of herbicides on an active pasture is problematic, cutting of the fern would be a measure which can warrant sustainable use of the *S. sphacelata* replanted pastures in South Ecuador. When growing undisturbed, *S. sphacelata* can suppress bracken by its competitive strength. Therefore it is the degree of weakening by grazing which decides about the sustainability of the pasture. This interrelation is at

present investigated in an experiment, in which different intensities of grazing are simulated by manual cutting.

REFERENCES

Ader KG (1990) The effect of climate and soils on bracken vigour and distribution on Scotland. In: Bracken Biology and Management (Thomas JA, Smith RT, eds) Austral Inst Agric Sci Occasional Publ. 40: 141-152.

Agee JK, Huff MH (1987) Fuel succession in a western hemlock/Douglas-fir forest. Canadian Journal of Forest Research 17: 697-704.

Beck E., Hartig K, Roos K (2008) Forest clearing by slash and burn. In: Beck E, Bendix J, Kottke I, Makeschin F and Mosandl R (eds.) Gradients in a Tropical Mountain Ecosystem of Ecuador. Ecological Studies, Vol. 198. Springer, Berlin Heidelberg, pp 371-374.

Burge M, Kirkwood R (1992) The control of bracken. Critical Reviews in Biotechnology 12: 299-333.

Carlisle A, Brown AHF, White EJ (1967) The nutrient content of tree stem flow and ground flora litter and leachates in a sessile oak (*Quercus petraea*) woodland. Journal of Ecology 55: 615-627.

Chen XQ, Wang JX, Tian HL, Zhang XC, Wen J, Zhou SL (2008) Development of microsatellite markers for the bracken fern, *Pteridium aquilinum*. Molecular Ecology Resources 8, 1491-1493.

Conway E, Arbuthnott M (1949) Occurrence of endotrophic mycorrhiza in the roots of *Pteridium aquilinum* Kuhn. Nature 163: 609-610.

Cox E, Marrs R, Pakeman R, Le Duc M (2008) Factors affecting the restoration of heathland and acid grassland on *Pteridium aquilinum*-infested land across the United Kingdom: A multisite study. Restoration Ecology 16: 553-562.

Daniels R (1985) Studies in the growth of *Pteridium aquilinum* (L.) Kuhn (bracken) .1. Regeneration of rhizome segments. Weed Research 25: 381-388.

De Bano, LR; Dunn, PH; Conrad, CE (1977) Fire's effect on physical and chemical properties of chaparral soils. In: Mooney HA; Conrad CE: Proceedings of the Symposium on the Environmental Consequences of Fire and Fuel Management in Mediterranean Ecosystems. USDA Forest Service General Technical Report WO-3. pp 65-74.

Der J, Thomson JA, Stratford J, Wolf P (2009) Global chloroplast phylogeny and biogeography of bracken (*Pteridium*; Dennstaedtiaceae). American Journal of Botany 96: 1041-1049.

Evans IA, Galpin O (1990) Bracken and leukaemia. Lancet 335: 231.

Farnworth J, Davies GM (1974) The response of hill bracken and the associated pasture to applications of picloram and dicamba. Weed Research 14, 401-404.

Fenwick GR (1989) Bracken (*Pteridium aquilinum*) – toxic effects and toxic constituents. Journal of the Science of Food and Agriculture 46: 147-173.

Flinn M, Pringle J (1983) Heat tolerance of rhizomes of several understory species. Canadian Journal of Botany 61: 452-457.

Hamer U, Potthast K, Makeschin F (2009) Urea fertilisation affected soil organic matter dynamics and microbial community structure in pasture soils of Southern Ecuador. Applied Soil Ecology 43: 226-233.

Hannam DAR (1986) Bracken poisoning in farm animals with special reference to the North York Moors. In: Smith, RT and Taylor, JA. Bracken: Ecology, Land Use and Control Technology. The Parthenon Publishing Group, Leeds Lancs, pp 133-138.

Hartig K, Beck E (2003) The bracken fern (*Pteridium arachnoideum*) dilemma in the Andes of South Ecuador. Ecotropia 9, 3-13.

Ingram DC (1931) Vegetative changes and grazing use on Douglas-fir cut-over land. Journal of Agricultural Research 43: 387-417.

MacLean DA, David A, Wein RW (1977) Nutrient accumulation for postfire jack pine and hardwood succession patterns in New Brunswick. Canadian Journal of Forest Research 7: 562-578.

Marrs R, Johnson S, Le Duc M (1998) Control of bracken and restoration of heathland. VI. The response of bracken fronds to 18 years of continued bracken control or 6 years of control followed by recovery. Journal of Applied Ecology 35: 479-490.

Page C (1982) The history and spread of bracken in Britain. Proceedings of the Royal Society of Edinburgh 81: 3-10.

Page C (1986) The strategies of bracken as a permanent ecological opportunist. In: Smith, RT and Taylor, JA. Bracken: Ecology, Land Use and Control Technology. The Parthenon Publishing Group, Leeds Lancs, pp. 173-181.

Pakeman RJ, Le Duc MG, Marrs RH (1998) An assessment of aerially applied asulam as a method of long-term bracken control. Journal of Environmental Management 53: 255–262.

Pakeman RJ, Le Duc MG, Marrs RH (2002) Bracken control, vegetation restoration and land Management. Food & Rural Affairs, Rural Development Service Technical Advice Note 1.

Parks JC, Werth, CR (1993) A study of spatial deatures of clones in a population of bracken fern, *Pteridium aquilinum* (Dennstaedtiaceae). American Journal of Botany 80: 537-544.

Roberts J, Pymar CF, Wallace JS et al. (1980) Seasonal changes in leaf area, stomatal and canopy conductances and transpiration from bracken below a forest canopy. Journal of Applied Ecology 17: 409-422.

Sharik TL, Ford RH, Davis ML (1989) Repeatability of invasion of Eastern White Pine on dry sites in Northern Lower Michigan. Am Midl Naturalist 122: 133-141.

Sheffield E, Wolf PG, Haufler CH (1989) How big is a bracken plant. Weed Research 29: 455-460.

Sheffield E, Wolf PG, Rumsey FJ, Robson DJ, Ranker TA, Challinor SM (1993) Spatial-distribution and reproductive-behavior of a triploid bracken (*Pteridium aquilinum*) clone in Britain. Annals of Botany 72: 231-237.

Snow CSR, Marrs RH (1997) Restoration of *Calluna* heathland on a bracken *Pteridium*-infested site in north west England. Biological Conservation 81: 35–42.

Speer WD (2008) Phylogenetic and biogeographic relationships among North American and Hawaiian *Pteridium aquilinum* (L.) Kuhn (Dennstaedtiaceae) based on chloroplast *rps4* and *rps4-trnS* intergenic spacer sequences. American Fern Journal 98: 179-193.

Speer WD, Werth CR, Hilu KW (1999) Relationships between two infraspecific taxa of *Pteridium aquilinum* (Dennstaedtiaceae). II. Isozyme evidence. Systematic Botany 23: 313-325.

Stewart GH (1988) The influence of canopy cover on understory development in forests of the Western Cascade Range, Oregon, USA. Vegetatio 76: 79-88.

Stewart G, Cox E, Le Duc M, Pakeman R, Pullin A , Marrs R (2008) Control of *Pteridium aquilinum*: Meta-analysis of a multi-site study in the UK. Annals of Botany 101: 957-970.

Tempel A (1981) Field studies of the relationship between herbivore damage and tannin concentration in bracken (*Pteridium aquilinum* Kuhn). Oecologia 51: 97-106.

Thomson J (2000) Morphological and genomic diversity in the genus *Pteridium* (Dennstaedtiaceae). Annals of Botany 85: 77-99.

Thomson JA (2008) Morphotype and conflicting taxonomies in *Pteridium* (Dennstaedtiaceae: Pteridophyta). Fern Gazette 18: 101-109.

Tryon RM (1941) A revision of the genus *Pteridium*. Rhodora 43: 1-31 and 37-67.

Veerasekaran P, Kirkwood RC, Fletcher WW (1977) Studies on mode of action of asulam in bracken (*Pteridium aquilinum* L. Kuhn). 1. Absorption and translocation of [asulam-c-14]. Weed Research 17: 33-39.

Veerasekaran P, Kirkwood RC, Fletcher WW (1978) Studies on mode of action of asulam in bracken (*Pteridium aquilinum* L. Kuhn). 3. Longterm control of field bracken. Weed Research 18: 315-319.

Waring RH, Major J (1964) Some vegetation of the Californian coastal redwood region in relation to gradients of moisture, nutrients, light, and temperature. Ecological Monographs 34: 167-215.

West TM, Richardson WG (1985) Response of bracken (*Pteridium aquilinum*) in pots to various herbicide treatments. Tests of Agrochemicals and Cultivars 6: 108-109.

Williams GH, Foley A (1975) Effect of herbicides on bracken rhizome survival. Annals of Applied Biology 79: 109-111.

CHAPTER 3

Species composition and genetic diversity

Species composition and genetic diversity of tropical bracken invading pasture land in southern Ecuador

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ABSTRACT

The rhizomatous bracken (*Pteridium aquilinum* s.l.) is one of the most aggressive weeds worldwide. Taxonomically, it represents a complex of subspecies and species, which has been partitioned into a northern and a southern, tropical clade. In this study, we analyzed the species composition of 50 samples of the bracken vegetation that invades and destroys pastures in the tropical Andes of South Ecuador. For identification we used allozyme analysis, sequencing of cpDNA, microsatellite data, and additionally morphological characters. Despite different resolution power of the applied methods, 29 samples were identified as *Pteridium arachnoideum*, 20 samples as *Pteridium caudatum*, and 1 as a potential hybrid. Using UPGMA-based tree clustering of D_{TL} distances as well as Bayesian clustering, similar relationships between the individuals of *P. arachnoideum* on the one hand and *P. caudatum* on the other could be shown. Comparison of observed and expected degrees of heterozygosity indicates higher gene flow and population stability for the diploid *P. arachnoideum* than for the allotetraploid *P. caudatum*, which at the study site reaches its upper altitudinal limit. Combining all methods of analysis, only 2 samples (*P. arachnoideum*) were identical, indicating either 2 samples from 1 individual or 2 ramets of the same genet. Because these samples were collected in a distance of 50 meters the extension of 1 individual or genet of the tropical species appears considerably smaller than that reported for the northern bracken. Ecological preferences of both *Pteridium* species could not be observed since both were found randomly distributed on the study site.

INTRODUCTION

Bracken (*Pteridium aquilinum* s.l., Dennstaedtiaceae) has proven one of the most successful and widespread vascular plant species worldwide (Harper 1982, Marrs & Watt 2006). Due to its particular morphological and physiological traits (Page 1976) it inhabits all continents exept Antartica. It responds positively to anthropogenic disturbance of the natural vegetation, especially of forests, and has become one of the worst weeds. Due to its life-form as a rhizomatous tall herb, studies of bracken

biology and bracken control have been mainly focused on the above ground organs, the fronds, which in the tropics can grow as high as four meters.

Taxonomically, bracken represents a complex of varieties or species and intermediate morphotypes, and its systematic structure is still controversially discussed (Tryon 1941; Burge & Kirkwood 1992, Thomson 2000, 2008; Marrs & Watt 2006, Der *et al.* 2009). In his revision of the genus *Pteridium*, Tryon (1941) used exclusively morphological characters. He considered bracken as a monotypic species comprising two subspecies and twelve varieties. However, because of a high phenotypic plasticity and high potential for intermediates and apomixis, morphological characters are not sufficient for a clear differentiation of species. Thus, molecular markers represent a promising additional tool for the identification of specimens and for the assessment of intermediate morphotypes.

Isoenzyme patterns have been used for taxonomic and population studies of bracken (Speer *et al.* 1999, Wolf *et al.* 1988, 1991, Korpelainen 1995), and also to investigate spatial extension of clones (Sheffield *et al.* 1989, Parks & Werth 1993). Another approach was based on chloroplast sequences (Speer 1999, 2008; Der *et al.* 2009). The uniparentally inherited, nonrecombinant nature of the plastome makes chloroplastic DNA sequences useful tools. Der *et al.* (2009) presented a new global taxonomic revision of the genus *Pteridium* based on the analysis of chloroplast rps4 and rps4-trnS intergenic spacer sequence variations. The authors distinguished two major clades: i) a primarily northern hemispheric (Laurasian/African) clade which includes the following subspecies of *P. aquilinum*: *pseudocaudatum*, *latiusculum*, *japonicum*, *pinetorum*, *decompositum*, *pubescens*, *wightianum*, *aquilinum*, *capense*, *centrali-africanum*, and *P. caudatum* (in part), and ii) a primarily southern hemispheric (Austral/South American) clade which includes *P. esculentum*, *P. arachnoideum*, *P. semihastatum* and *P. caudatum* (in part). Two of these species, *P. caudatum* and *P. semihastatum*, are *allo*-tetraploid taxa with putative progenitors in the northern and southern clades (Thomson, 2000; Thomson & Alonso-Amelot, 2002; Der *et al.*, 2009). Hybridization and allopolyploidy occurs frequently in ferns in adaptation to changing environmental conditions (Page, 2002).

Microsatellite analysis is one of the most promising molecular tools to identify and differentiate individuals, varieties and species. The co-dominant inheritance of microsatellites, the high level of polymorphism and reproducibility, and also easy handling make them very useful for many approaches (Chavez Galarza *et al.* 2010, Erre *et al.* 2010, Hirai *et al.* 2010, Pegueroles *et al.* 2010). Hitherto, these markers have been rarely used in ferns, but recently Chen *et al.* (2008) published microsatellite markers for bracken which could allow higher resolution of population structures.

In the humid tropical Andes of South Ecuador, where the present study has been conducted, bracken plays an enormous economical role as an aggressive invader of pastures (Hartig & Beck 2003, Beck *et al.* 2008, Göttlicher *et al.* 2009) which provide the livelihood of most of the local people. By morphological inspection, two bracken species, *Pteridium arachnoideum* (KAULF.) MAXON and *Pteridium caudatum* (L.) MAXON could be tentatively identified as invaders. The two species have overlapping distribution areas within northern South America and Central America (Tryon & Tryon 1982) and the diploid *P. arachnoideum* is considered as one progenitor of the allotetraploid *P. caudatum* (Thomson & Alonso-Amelot 2002, Der *et al.* 2009). Morphological differentiation is by the shape of the pinnule segments next to midvein of the fronds (Alonso-Amelot & Rodulfo-Baecher 1996). A separate, small lobule next to the basis of the pinnae and pinnules is typical of *P. arachnoideum*, whereas the segments of *P. caudatum* broaden toward their base at the midrib, termed “decurrent” by Tryon (1941).

Since in bracken the generation of new ramets is by clonal propagation through fragmentation of rhizomes, molecular methods offer easy analysis of genet extension without excavating the rhizomes. From allozyme studies in North America and the UK, Sheffield *et al.* (1989) and Parks & Werth (1993) concluded that bracken genets could have dimensions of 390 meters up to over 1,000 meters. In the humid tropics, where growth is not interrupted by a winter break, even larger areas of one bracken genet may be expected. Although some authors (Wolf *et al.* 1988, Korpelainen 1995) supposed underestimation of the number of genotypes and

concomitant overestimation of the area of one genet due to the limited resolution of allozyme analysis, these data are still used for assessing genet variability and extension.

In the study presented here, we used allozyme analysis, sequencing of the chloroplast rps4 and rps4-trnS intergenic spacer region, and microsatellite data in addition to morphological characters for a clear identification of the invader species and their potiential hybrids on a pasture in southern Ecuador. Furthermore, we aimed to assess the distribution of the species on the study area and the extension range of an individual or genet.

MATERIAL & METHODS

Sampling site

The study area is located on the steep northern slopes of the narrow upper Rio San Francisco valley in the eastern Andes of South Ecuador (3°58'30'' S, 79°4'25'' W, Fig. 3-1A). The perhumid climate is characterised by an almost constant annual temperature of 15.5 °C and an annual precipitation of 2050 mm (Bendix *et al.*, 2008). Parent rock material consist of various silicious minerals and the soils are predominantly cambisols (Beck *et al.*, 2008), with a shallow, sometimes even lacking organic layer. The southern slopes of the river valley are part of the forested Podocarpus National Park whereas on the northern slopes the mountain rain forest has been cleared about fifty years ago for pasture farming. However, in the meantime, 40% of the former pastures have been abandonded because of infestation by bracken (Göttlicher *et al.* 2009). These areas are covered by a vegetation of bracken, up to two meters high, with scattered bushes and remnants of the former pasture grasses. A heavily bracken-infested area of 19.25 ha between 1,800 and 2,100 m above sea level was chosen for detailed analysis of the population structure of this weed.

Sampling Procedure

Much larger genet extentions than at the northern hemisphere were expected in the research area due to year-round growing season. For random sampling a virtual grid was placed on the research area with grid cells of 50 × 50 m. Mature fronds were sampled from each junction of the grid wherever the terrain allowed access. The samples were kept in a cool box for transportation to the laboratory. One portion of each sample was stored in the refrigerator until allozyme analysis in the laboratory of the Center of Cellular & Molecular Biology of the Universidad Técnica Particular de Loja (UTPL) in Ecuador. The other portion was frozen at -80°C and analyzed in the DNA Analytics & Ecoinformatics laboratory of the University of Bayreuth in Germany.

Morphological identification

The particular species was determined by the occurrence of free lobes between pinnules. Fronds with free lobes were considered as *P. arachnoideum*, those with a broadening basis of the pinnules as *P. caudatum*. Intermediates could not been associated with one of the species.

Allozyme analysis

For allozyme analysis we chose six enzymes, phosphoglucoisomerase (PGI), esterase (EST), menadione reductase (MNR), isocitrate dehydrogenase (IDH), malate dehydrogenase (MDH) and triose-phosphate isomerase (TPI). Using horizontal starch gel electrophoresis (Müller-Starck *et al.* 2001) these showed clear polymorphic banding patterns (Tab. 3-1). Samples of 30 – 35 mg fresh weight (equivalent to 2 – 4 pinnules) were homogenized in 90 µl extraction buffer (0.13 M Tris, 0.004 M EDTA, 3% PVP, and 1% 2-mercaptoethanol, pH 7.3) to which a trace of quartz sand and PVPP was added. For quick homogenization, we used the wells (9 mm diameter) of a multi-well acryl plate as minimortars and an acryl rod as pestle

that was excentrically fixed on an electric motor. Homogenization was for 20 s while the minimortar plate was cooled from below. The homogenates were resorbed by rectangular pieces of 3MM Whatmann paper and stored overnight at -20°C. Starch (GERBU, Gaiberg, Germany) was dissolved by boiling in the appropriate buffers (Tab. 3-1): 9.5% in gel buffer no. 3, or 10% in gel buffers no. 1 and 2. Glucose (2%) was added to gel buffers no. 2 and 3. The 0.5 cm thick gels were stabilized with urea (1%). Gels were loaded from the soaked paper pieces and electrophoresis was carried out as detailed in Tab. 3-1. The horizontally layered gels were sliced with a metal thread into 1.3 mm thick slabs and treated with the special buffers for enzyme detection. Enzyme reactions and visualization was performed using the methods of Müller-Starck et al. (2001) for IDH, MDH, PGI, EST, and MNR. TPI detection was according to Soltis *et al.* (1983).

Tab. 3-1: Experimental details of starch gel electrophoresis for allozyme analysis according to Müller-Starck *et al.* 2001 (modified as listed in the Table).

Enzyme system	*E.C. #*	*Prebuffer*[§]	*Electrode/ gel buffer**	*Gene locus*
Isocitrate dehydrogenase (NADP)	1.1.1.42	Tris-HCl	1	IDH-A
Malate dehydrogenase (NAD)	1.1.1.37	Tris-HCl	1	MDH-B
Phosphoglucose isomerase	5.3.1.9	Tris-HCl	2	PGI-A
Triose-phosphate isomerase	5.3.1.1	Tris-HCl	1	TPI-B
Esterase	3.1.1…	Phosphate	3	EST-A, -B
Menadione reductase	1.6.99.2	Tris-HCl	3	MNR-B

[§] Prebuffers:

Tris-HCl buffer: 0.08 M Tris pH 8.0 adjusted with HCl
Phosphate buffer: 0.2 M NaH_2PO_4 and 0.16 M Na_2HPO_4 prepared separately; shortly before use mixed with NaH_2PO_4 : Na_2HPO_4 = 2.5 : 1

* Electrode and gel buffers, and conditions for electrophoresis

No.	*Electrode buffer/ pH*	*Gel buffer/ pH*	*Current [mA]*	*Running time [h]*
1	0.14 M Tris, 0.043 M citric acid/ 7.5	Electrode buffer : H_2O = 1 : 2.5	75	11
2	0.19 M boric acid, 0.05 M LiOH/ 8.5	0.05 M Tris, 0.008 M citric acid + 10% electrode buffer/ 8.1	60	3.5
3	0.3 M boric acid, 0.06 M NaOH/ 8.0	0.07 M Tris pH adjusted with 1M HCl + 3% electrode buffer/ 8.7	70	4

Nucleic acid extraction

Frozen leaf pieces (40 – 70 mg, 1 – 2 cm^2) were blended in 200 µl extraction buffer (NucleoMag 96 Plant kit; Machery-Nagel, Düren, Germany, containing 5 µl RNAse A) with a FastPrep®-24 Tissue Homogenizer (MP Biomedicals Europe, Illkirch, France) for 40s at a speed of 6m/s. Insolubles were pelleted at 15,000 x g for 5 min at room

temperature. Genomic DNA was prepared from the supernatant using the NucleoMag 96 Plant kit adapted to the KingFisher automated purification system (Thermo Scientific, Langenselbold, Germany). Details of the nucleic acid purification procedure are presented in Tab. 3-S1 of the supporting information. The purified genomic DNA was diluted tenfold and used for all subsequent PCR reactions.

Sequencing of the chloroplast rps4-trnS marker

For species identification, fragments of the chloroplast ribosomal small subunit-4 protein (rps4) and the rps4-trnS intergenic region were amplified using oligonucleotide primers (Speer 1999, 2008). Since PCR with some of these primers resulted in multiple bands, additional oligonucleotide primers were designed for conserved regions in an alignment of rpS5-trnS sequences of *Pteridium* from the NCBI nucleotide database. With a combination of primer M13R-RPS5F (the original RPS5F primer modified by the addition of a M13 reverse primer site at the 5' end) and a new primer *Pteridium*-R2 (5'-GTATGCCAAACCAACTTG) specific cpDNA fragments from total DNA preparations were amplified. PCR fragments were purified with the AMPure system, sequenced using the GenomeLab Dye Terminator Cycle Sequencing kit, and analysed on a GenomeLab GeXP Genetic Analysis System (all components from Beckman-Coulter, Krefeld, Germany).

Microsatellite analysis

To investigate whether bracken microsatellite primers developed by Chen *et al.* (2008) can be used to amplify fragments in bracken samples from Ecuador, a preliminary primer screening was performed for the eight described loci (seven in the nuclear plant genome and one in the plastome). Five of the primer pairs turned out as suitable for allele calling (locus Pter03, Pter04, Pter06, Pter09, Pter12; Tab. 3-3). For large-scale analysis, two multiplex PCRs were designed to amplify fragments of three and two different microsatellite loci, respectively, in a single reaction. In addition, the relative amount of specific primers was adjusted experimentally in each

multiplex design to equalize large differences in PCR product amounts. The final concentration and dyes used as labels for each microsatellite primer pair were for Multiplex I: Pter03 (0.04 µM, labelled with BMN-5), Pter04 (0.08 µM, labelled with DY-751), and Pter09 (0.2 µM, labelled with BMN-6); for Multiplex II: Pter06 (0.2 µM labelled with DY-751) and Pter12 (0.04 µM, labelled with BMN-6). In both multiplex reactions, the forward primers were labelled at the 5'-end with any of the fluorescent dyes. A 25 µl microsatellite PCR contained 0.5 µl of tenfold diluted DNA, microsatellite primers, and Multiplex PCR Mix containing Q-solution (Qiagen, Hilden, Germany). Multiplex PCR products were diluted tenfold and separated on a capillary electrophoresis system (CEQ8000, Beckman-Coulter, Krefeld, Germany). Electrophoretic patterns were examined by GeneMarker v1.90 (Softgenetics, PA, USA).

Data analysis

Interpretation of banding pattern. Ploidy classification was indirectly through electrophoretic banding patterns in the allozyme and microsatellite analysis. Allozyme patterns were analysed according to Hardy *et al.* (2000) considering banding intensity as additional information. Microsatellite pherograms were also analysed by allele mapping, but banding intensity was not taken into account. If at least two microsatellite and/or allozyme loci showed allele genotypes with three or four alleles, this individual was classified as tetraploid while one- or two-allelic genotypes were treated as diploid.

Species differentiation. The method of Tomiuk & Loeschcke (1991) was used, to calculate genetic distances (D_{TL}) between all samples for allozyme, microsatellite, and morphological data, individually and in combination using the program Popdist 1.2.0 (Guldbrandtsen et al. 2009), which can handle also polyploid species. To avoid biased calculations (due to clustering of individuals at ploidy level) all samples were standardized to tetraploid level by filling the additional digits with "0" if only one, two, or three alleles at one locus were recorded. To construct UPGMA based trees, D_{TL} data were processed in "Neighbor" from the program package Phylip 3.69

(Felsenstein 2006) and subsequently viewed with Treeview 1.6.6. (Page 1996). To verify the goodness of fit of the UPGMA analyses, the cophenetic correlation coefficients (r_C) were computed (Dighe et al. 2004) using the similarity matrix of Popdist and the tree output matrix of Neighbor.

Bayesian structure analysis. To infer the genetic structure and admixture of the samples, a Bayesian cluster analysis was performed using the program BAPS 5.3 (Corander *et al.* 2003, 2008, 2009). Data input was based on tetraploid banding pattern as for Popdist. First, all individual samples were clustered without predefinition of species to ensure independent clustering using data of morphological, allozyme, and microsatellite analysis individually and in combination. To find the optimal clustering, the calculations were done several times with input of the maximum number of populations/groups ranging from 2 to 10. Subsequently, admixture was calculated based on the optimal mixture clustering. The following default values were used for the simulations: i) 100 iterations to estimate the admixture coefficients of individuals, ii) 200 reference individuals from each population, and iii) 20 iterations to estimate admixture coefficients of reference individuals. The calculated admixture coefficients of each sample represent a Bayesian posterior mean estimate, which gives the estimated proportion of the genome associated with the clusters. Genetic distances between the clusters were calculated based on Nei's distances (all loci).

Genetic diversity. Allelic diversity was determined as allele frequency and determined as the total number of allozyme and microsatellite alleles per locus in general and for the two species (based on previous clustered individuals). Allele frequency differences between the two species were calculated as the proportion of unique alleles. Genetic diversity (expected heterozygosity corrected for sample size, H_{EC}) was calculated according to Nei (1987) based on 10,000 Monte Carlo (MC) simulations with:

$$H_Ec_{di} = \frac{2n}{2n-1}\left(1 - \sum_{i=1}^{s} p_i^2\right) \quad \text{for the diploid species} \quad [1]$$

and

$$H_Ec_{te} = \frac{4n}{4n-1}\left(1 - \sum_{i=1}^{s} p_i^2\right) \quad \text{for the tetraploid species,} \quad [2]$$

where p_i is the frequency of the i^{th} allele at a given locus in a population, n the sample size and s the number of genotypes. Observed heterozygosity (H_O) was calculated as the proportion of individuals showing heterozygosity at a locus. Evenness was used as an indicator for the distribution of genotypes at different loci within the populations of *P. arachnoideum* and *P. caudatum*, respectively and was calculated as:

$$even = \frac{1}{s}\,\frac{1}{\sum_{i=1}^{s} p_i^2} \quad [3]$$

where s is the number of genotypes. An evenness of 1 indicates equal frequencies of all included genotypes. For calculations of H_Ec_{te} and evenness the program ATETRA 1.2.a (Van Puyvelde et al. 2010) was used. To calculate H_Ec_{di}, H_E of the samples clustered as *P. arachnoideum* was calculated by ATETRA and was corrected manually for sample size.

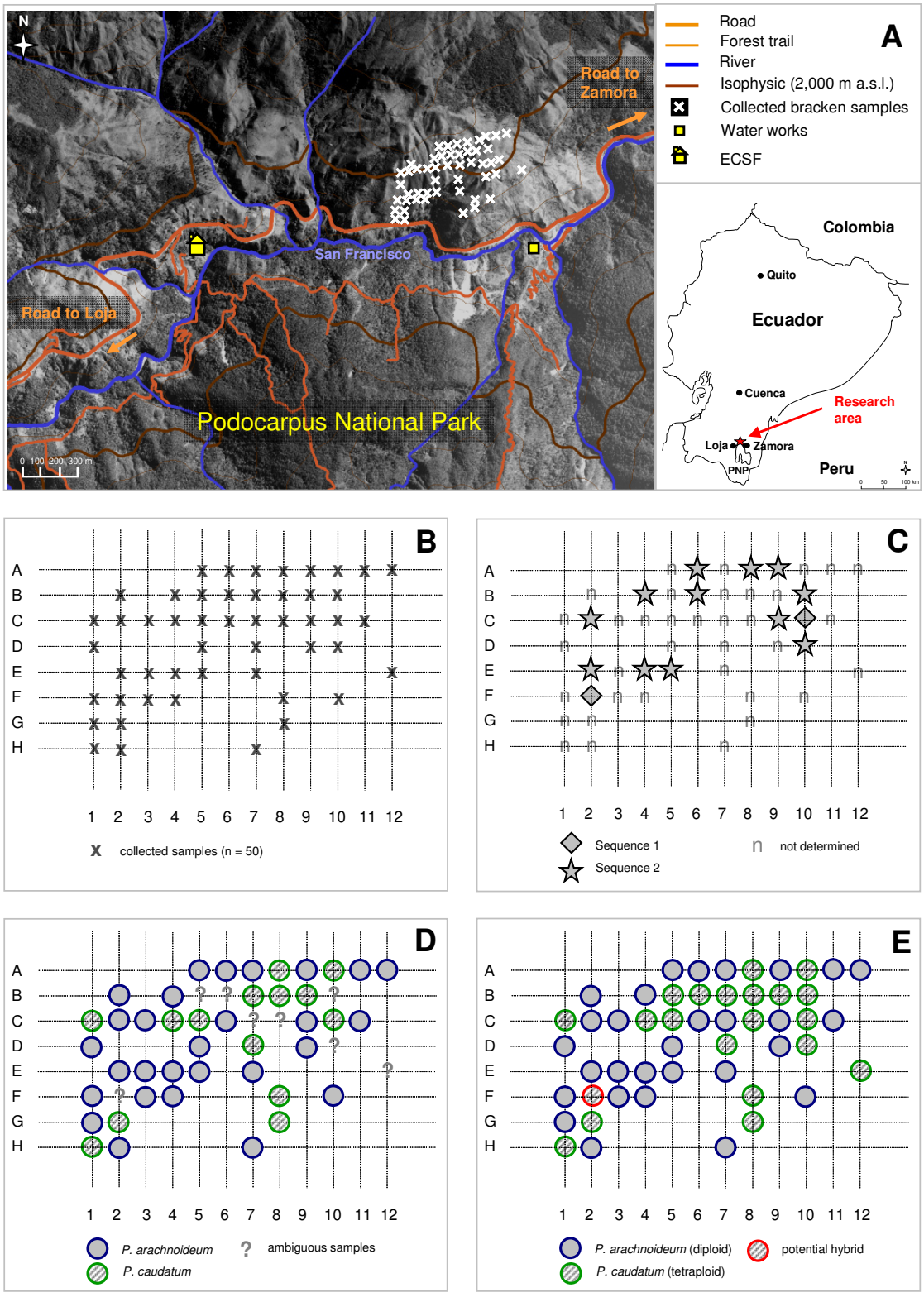

Fig. 3-1: A, Location of the research area in Ecuador and of the study site in the upper San Francisco valley (PNP = Podocarpus National Park, ECSF = research station "Estación Científica San Francisco"); B, sampling grid; C, results of the rps4-trnS sequencing, D, identification of samples by morphological characters only; E, distribution of bracken species on the study site. For identification of the samples all available data were compiled.

RESULTS

Classification of bracken samples

Applying the various methods of species identification on the bracken vegetation of the study area showed the usefulness of the morphological characters by which *P. arachnoideum* and *P. caudatum* could be unambiguously identified in 84% of the samples. By observing the presence or absence of free lobes between the segments, 28 samples were identified as *P. arachnoideum* and 14 as *P. caudatum* (Fig. 3-1D). Eight samples could not be associated with one of the species by morphological inspection alone as they showed characteristics of both bracken species, which means that all over the frond partly free lobes were found and partly not.

Ploidy of the samples and in turn the species was identified indirectly on the basis of banding pattern of allozyme and microsatellites (number of alleles per locus, Tab. 3-S2 and 3-S3 of supporting information), assuming that the diploid samples represent *P. arachnoideum* and the tetraploid *P. caudatum* (Thomson & Alonso-Amelot 2002, see Fig. 3-1E). The results obtained with this method confirmed the morphological classification (Fig. 3-1D). In addition, seven of the eight individuals, which could not be clearly identified by morphological characters, were genetically identified as tetraploid samples (B5, B6, B10, C8, D10, E12, F2; Fig. 3-1E).

Sequencing of chloroplast rps4 and rps4-trnS intergenic spacer region resulted in two different DNA motifs with six variable sites and a size of 550 - 600 bp. According to Der *et al.* (2009), sequence 1 conforms with the GenBank accessions FJ177141, FJ177142, and FJ177144 belonging to *P. caudatum* from Costa Rica, Columbia, and Venezuela. Sequence 2 conforms with accessions FJ177140, FJ177138, and FJ177139 representing *P. arachnoideum* from Mexico, Brazil, and Venezuela, and FJ177143 belonging to *P. caudatum* from Costa Rica. Fourteen of our samples were analysed using the corresponding primers (Fig. 3-1C). Two samples showed sequence 1, which was also confirmed as *P. caudatum* by the other

molecular analyses. Eight out of twelve samples corresponding to sequence 2 were identified by allozyme and microsatellite allele patterns as *P. arachnoideum* and the remaining four as *P. caudatum*. The two obtained motifs found within the *P. caudatum* samples, possibly indicate different populations of this species. Since the use of the chloroplast rps4 and rps4-trnS intergenic spacer region showed not the expected detailed information regarding species identification, further sequencing of the remaining samples was not performed.

Clustering of samples

As mentioned above, the occurrence of up to two alleles over all loci was considered an indicator for diploidity and, thus, for the species *P. arachnoideum*, although homozygot tetraploid samples (of the species *P. caudatum*) could be assumed to show the same allele pattern. This possibility could largely be ruled out by using standardized tetraploidy for clustering approaches. Morphological, allozyme, and microsatellite data was used to assess relationships among individuals. The dendrogram topology based on calculated pairwise genetic distances of allozyme and microsatellite data, respectively, showed a clear separation into two and three main branches, accordingly. The classification coincided with the previous separation of diploid and tetraploid samples as indicated by the banding patterns of allozyme and microsatellite analysis. The second and third main branch of microsatellite data consisted of tetraploids. The radial UPGMA based tree of the combined morphological and molecular data is shown in Fig. 3-2 (with $r_C = 0.79$, indicating a good fit of the presented tree with the genetic distance matrix). The 50 samples clustered into two main branches, which was in agreement with previous species identification (Fig. 3-1E). The sample F2 was the only exception, which, in spite of a tetraploid banding pattern in the microsatellite analysis, was allocated to *P. arachnoideum*. The samples E3 and F3 showed a similarity value of 1, indicating that these two samples were collected from one individual or genet in a distance of 50 meters (minimal collection distance).

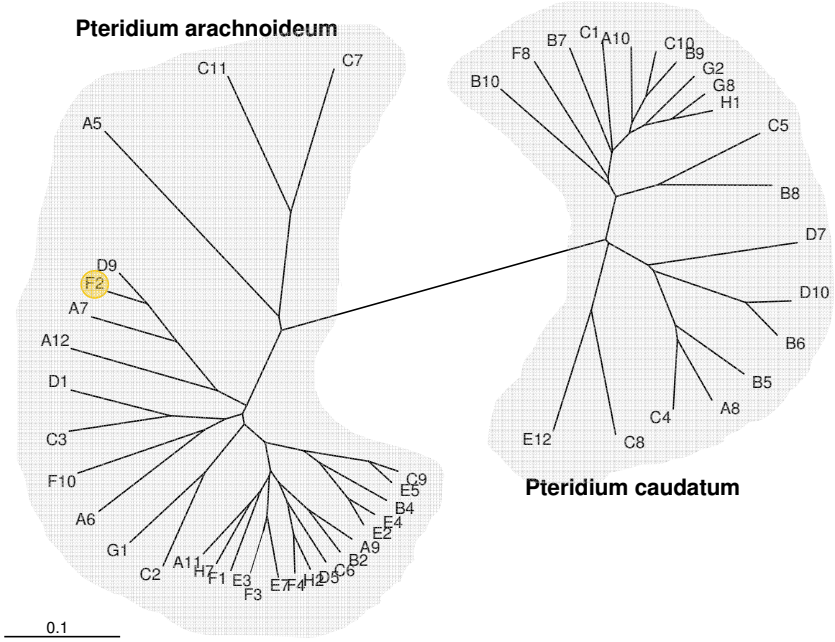

Fig. 3-2: UPGMA tree based on D_{TL} distances of combined allozyme (7 loci), microsatellite (5 loci) and morphology data showing the over-all relationship of all investigated individuals (n = 50). Marked sample F2 was classified as tetraploid by molecular data.

Also Bayesian analysis based on combined morphological and molecular data revealed an optimal partitioning in two clusters, the *P. arachnoideum* and the *P. caudatum* cluster with 30 and 20 samples, respectively, with the same aggregation of samples as in the UPGMA based tree in Fig. 3-2. Significant admixture between clusters was found only with sample F2 with an admixture coefficient of 0.29 for the cluster “caudatum” and 0.71 for the cluster “arachnoideum”. All other samples had coefficients of 1 or 0, indicating no admixture. Nei’s genetic distance between the clusters was 0.18 (calculated with BAPS). Separate clustering of the samples belonging to *P. caudatum* and *P. arachnoideum*, respectively, revealed no further subclusters as could be expected especially for *P. caudatum*, because of the two chloroplast sequences of the rps4 and rps4-trnS intergenic spacer region.

The sample F2 showed tetraploid banding pattern in the microsatellite analysis, but diploid banding pattern in the allozyme analysis. Based on combined morphological and molecular properties, it was clustered into the “arachnoideum aggregate” using two different methods, and it is the only sample which showed

admixture with the “caudatum aggregate”. Thus, F2 may be a possible hybrid between these two species and thus was excluded from the following calculations.

Genetic variability of allozyme loci

The total number of allozyme genotypes was 22 for the samples classified as *P. arachnoideum* (out of 29) and 18 for *P. caudatum* (out of 20). Nine samples of the locus EST-A showed no bands. In that case absence of bands was considered as null alleles. H_O ranged between 3% and 57% for *P. arachnoideum* (average 20%) and between 0% and 75% for *P. caudatum* (average 27%). H_Ec_{di} was between 3% and 72% with an average of 21%, and H_Ec_{te} was between 0% and 62% with an average of 39% (Tab. 3-2). Evenness was very low for the diploids and only little higher for the tetraploids (Tab. 3-2). Some alleles found in *P. arachnoideum* were not present in *P. caudatum* and vice versa (MNR-B/2; EST-B/3, MDH-B/2, TPI-B/1, IDH-A/2; Fig. 3-3). At locus MNR-B, the allele 2 occurred in the tetraploid species with a frequency of 0.9 but could not be detected in the diploid samples. All other mentioned alleles showed frequencies lower than 0.75. The proportion of allozyme alleles found in *P. arachnoideum* but not in *P. caudatum* was 12%. Unique for *P. arachnoideum*, these alleles showed very low frequencies (MDH-B/2, TPI-B/1; Fig. 3-3). The reverse case, the proportion of alleles found in *P. caudatum* but not in *P. arachnoideum*, was 17%. Two of these alleles occurred with a high frequency (0.74 and 0.9, respectively).

Tab. 3-2: Characteristics of seven allozyme loci from *Pteridium arachnoideum* (a) and *Pteridium caudatum* (c). H_O: observed heterozygosity; H_Ec expected heterozygosity corrected for sample size

Locus	*Number of alleles*			H_O		H_Ec		*evenness*	
	all	*a*	*c*	*a*	*c*	*a* (H_Ec_{di})	*c* (H_Ec_{te})	*a*	*c*
MNR-B	2	2	2	0.03	0	0.03	0.49	0.03	0.10
EST-A	2	2	2	0.13	0	0.13	0.22	0.04	0.11
EST-B	3	2	3	0.17	0.75	0.16	0.62	0.04	0.13
PGI-A	4	4	4	0.57	0.40	0.72	0.40	0.11	0.08
MDH-B	3	3	2	0.20	0.55	0.19	0.49	0.04	0.10
TPI-B	2	2	1	0.13	0	0.13	0	0.04	0.05
IDH-A	3	2	3	0.20	0.20	0.14	0.54	0.04	0.11
Total/ Average	19	17	18	0.20	0.27	0.21	0.39	0.05	0.10

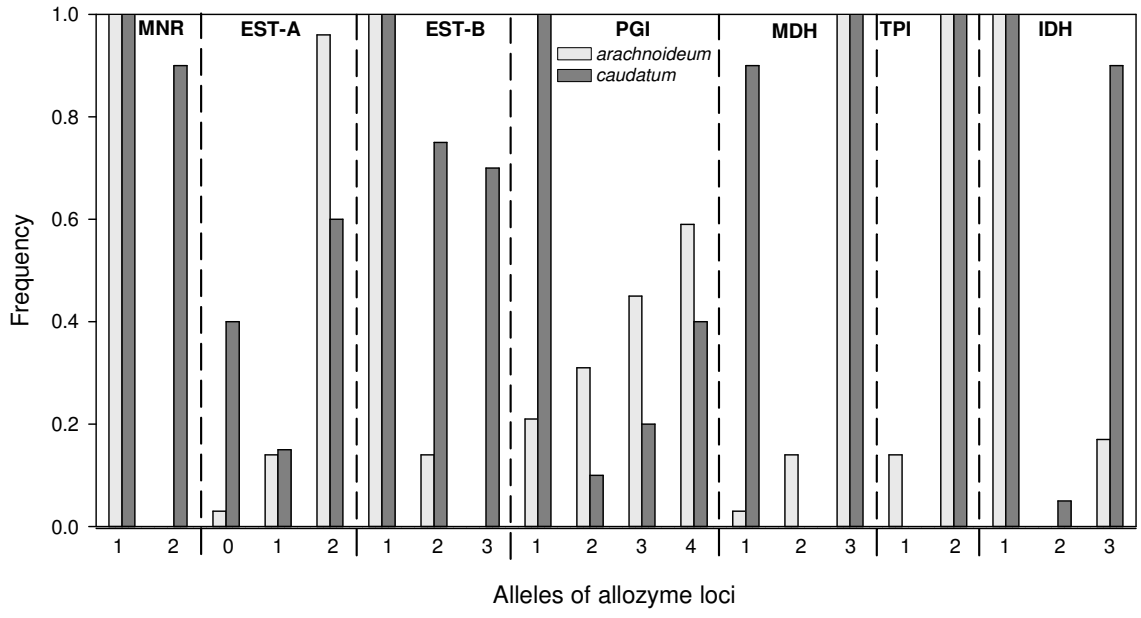

Fig. 3-3: Allele frequencies of 7 allozyme loci for *P. arachnoideum* and *P. caudatum*. For one individual a present allele was counted only once independently of zygosity or ploidy level.

Genetic variablity of microsatellite loci

The total number of different genotypes was 48 out of 49 samples. Two samples of *P. arachnoideum* (E3 and F3) were identical (see above). The five analysed microsatellite loci were highly polymorphic with on average 15.4 (± 9.6 SD) alleles per locus. The samples of both species showed similar polymorphism levels with 10.8 (± 7.4 SD) alleles per locus for *P. arachnoideum* and 10.0 (± 5.8 SD) alleles per locus for *P. caudatum* (for detailed information per locus see Tab. 3-3). H_O was between 10% and 93% for *P. arachnoideum* (average 61%) and between 0% and 90% for *P. caudatum* (average 48%). H_Ec_{di} was between 11% and 94% with an average of 62%, and H_Ec_{te} was between 60% and 90% with an average of 77% (Tab. 3-3). The distribution of genotypes at the different microsatellite loci was inhomogeneous (evenness between 0.04 and 0.47, Tab. 3-3). This was also reflected by the distribution of allelic frequencies, which was inequal within the analysed loci with one to three common alleles per locus and many rare ones (details in supporting information Tab. 3-S4). The alleles 174 bp of Pter03 and 206 of Pter06 showed comparable high frequencies in both species. At locus Pter04, one allele with 217 bp size was common in all samples of *P. caudatum*, but never present in *P. arachnoideum*. Therefore, the occurrence of this allele is useful for species differentiation. The proportion of unique alleles among species was nearly 50% for each species. Null alleles were present with low frequencies at loci Pter03 and Pter12 of samples of *P. arachnoideum*.

Tab. 3-3: Characteristics of the five selected microsatellite loci from *Pteridium arachnoideum* (a) and *Pteridium caudatum* (c). H_O: observed heterozygosity; H_Ec expected heterozygosity corrected for sample size

Locus	*Motif*	*Forward primer sequence (5'-3')*	*Reverse primer sequence (5'-3')*	*Size (bp)*	*No. of alleles*			H_O		H_{EC}		*evenness*	
					all	*a*	*c*	*a*	*c*	*a* (H_{ECdi})	*c* (H_{ECte})	*a*	*c*
Pter03	$(TG)_{14}$	AAGATCAATCGCGGACAC	CATTCTCACTAACTACCCAC	174-272	17	11	8	0.70	0.90	0.62	0.80	0.10	0.24
Pter04	$(CA)_6$	ATCAAGCCAAGGTCAC	AACCCATGATTGCTAAT	213-257	13	5	12	0.57	0.85	0.71	0.84	0.11	0.30
Pter06	$(AG)_{19}$	CCTCCATTCTTGTCTCATTTATC	CTACTCATCTACCTGCTCTTGC	196-216	10	10	7	0.73	0	0.74	0.72	0.13	0.18
Pter09	$(CT)_8(GTCTCT)_3(CT)_{15}$	GGAGGTGGCTATTATTGT	CTCTTCTAGCAAGCAGGT	260-340	31	23	19	0.93	0.65	0.94	0.90	0.47	0.45
Pter12*	$(AT)_9$	TGGTGAAGTTGTGATGCCTAC	TATCGGTGGAAAGAAAGAGTG	360-374	6	5	4	0.10	0	0.11	0.60	0.04	0.12
Total/ Average					77	54	50	0.61	0.48	0.62	0.77	0.17	0.26

*located in the plastome

DISCUSSION

Species identification and admixture

With our small-scale analysis of southern bracken based on morphological and molecular data, we confirmed the occurence of the two tropical bracken species *Pteridium arachnoideum* and *Pteridium caudatum* growing side by side in the research area in South Ecuador. We showed that based on only one pair of morphological characters (presence/absence of free lobes between segments), differentiation of both species was possible in the majority of cases (84%). With additional analysis of other characters, like e.g. frond colour as used by Alonso-Amelot & Rodulfo-Baechler (1996), identification could not be improved, because of the fuzziness of these characters. Thus, for clear classification of the two species also with regard to the detection of possible hybrids, additional genetic information is needed. With the analysed seven allozyme and five microsatellite loci, respectively, clear species separation was achieved except for one sample (F2). Subsequent Bayesian clustering and quantification of admixture confirmed the separation into two clearly distinguishable species with uniform genetic structures. Admixture was revealed only for the mentioned sample F2, which was tentatively identified as hybrid. Hybridisation is widespread in Pteridophytes and intermediates have been described also for allopatric bracken species in overlapping ranges (Page 1976). Thomson (2000) reported an accession of *P. arachnoideum* from the Galapagos Islands (TGAL), which by the chromosome number ($4n = 208$) should be a tetraploid and thus was addressed as hybrid.

The chloroplast rps4 and rps4-trnS intergenic spacer region was less useful in species differentiation. Only two different sequences were found: sequence 1 which was clearly related with *P. caudatum* and sequence 2 which occured in both species. The same finding, however with only one sample from Costa Rica showing sequence 2, is reported by Der *et al.* (2009). Since the diploid *P. arachnoideum* is one of the putative progenitors of the tetraploid *P. caudatum* (Thomson & Alonso-Amelot 2002), this sequence seems to be highly preserved in the two species.

Intraspecific diversity

Based on allozyme analysis, H_O values of the two populations of *P. arachnoideum* and *P. caudatum*, respectively (H_O = 0.20 for *P. arachnoideum* and 0.28 for *P. caudatum*), were in the upper range of populations of other bracken species, such as *P. aquilinum* var. *latiusculum* (Desv.) Underw. (H_O = 0.10 - 0.21; Speer *et al.* 1999). On the other hand they were much higher than those of bracken populations from North America and Europe (H_O between 0 and 0.14, Wolf *et al.* 1988, Korpelainen 1995, Speer *et al.* 1999) which had been analysized by allozyme studies, too.

For the investigated population of *P. arachnoideum*, the H_O and H_Ec_{di} values, as determined with both methods, were rather similar. As far as it can be stated based on a sample size of 29 individuals, this similarity indicates considerable gene flow by ongoing sexual reproduction and, in turn a genetically stable population (Hardy *et al.* 2000). Sexual reproduction is triggered by the burning of the infested pastures by the farmers. Heat initiates sporulation on surviving fronds and subsequent ample gametophyte development on the bare and nutrient-rich ground which gives rise to enhanced sexual reproduction.

The average H_O values of allozyme and microsatellite loci of *P. caudatum* were lower than the average H_Ec_{te} indicating a strong heterozygote deficiency. Since both species share the same site, the effects of burning on the frequency of sexual reproduction should be similar. A smaller extent of gene flow, however, could be due to the fact that at the study site *P. caudatum* reaches the upper limit of its altitudinal range (Alonso-Amelot & Rodulfo-Baechler 1996), where its potential of sexual reproduction may be restricted. The increased variation in allelic diversity (as shown by the degree of H_Ec_{te}) on the other hand, is a result of its allotetraploidy (Soltis & Soltis 2000).

Estimation of individual genet size

Combining all methods of investigation, only two samples (E3 and F3) of *P. arachnoideum* were identical, indicating two ramets belonging to the same genet. These were collected in a distance of 50 meter. Due to the applied pattern of sampling, all other genotypes would have an extension of less than 50 meters. This is surprising because studies of bracken on the northern hemisphere reported genet extentions of several hundred (Sheffield *et al.* 1989) up to over thousand meters (Parks & Werth 1993). But the study of Parks & Werth (1993) was based on five allozymes with six polymorphic loci, thus, following our results, may be biased by insufficient genotype resolution. Underestimation of genetic variation by allozyme studies was also supposed by Wolf *et al.* (1988), but probably underestimated also in their own study. Sheffield *et al.* (1989) investigated more enzyme systems with up to 15 polymorphic loci resulting in a higher resolution of genotypes. Their largest clone had an extension of 390 meters. Our data of *P. arachnoideum* and *P. caudatum* based on microsatellite analysis suggest only small scale extension of genets. If this is an indication of repeated infestation by bracken spores triggered by repeated burning of the area, is still an open question.

The distribution of both bracken species over the area of nearly 20 ha was random, indicating no differential habitat preference or clustering of individuals of one of the species. The observed dominance of *P. arachnoideum* was due to the fact that *P. caudatum*, as a lowland species reaches its upper altitudinal limit. Thus, the particular proportions may be accidental.

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REFERENCES

Alonso-Amelot, ME & Rodulfo-Baechler, S 1996, 'Comparative spatial distribution, size, biomass, and growth rate of two varities of bracken fern (*Pteridium aquilinum* L. Kuhn) in an neotropical montane habitat', Vegetatio (Plant Ecology) vol. 125, pp 137-147.

Beck, E, Makeschin, F, Haubrich, F, Richter, M, Bendix, J, & Valerezo, C 2008, 'The ecosystem (Reserva Biológica San Francisco)', In: Beck E, Bendix J, Kottke I, Makeschin F & Mosandl R (eds.) *Gradients in a Tropical Mountain Ecosystem of Ecuador*. Ecological Studies, vol. 198. Springer, Berlin Heidelberg, pp 1-13.

Bendix, J, Rollenbeck, R, Richter, M, Fabian, P, & Emck P 2008, 'Climate', In: Beck E, Bendix J, Kottke I, Makeschin F and Mosandl R (eds.) *Gradients in a Tropical Mountain Ecosystem of Ecuador*. Ecological Studies vol. 198. Springer, Berlin Heidelberg, pp 63-73.

Burge, MN & Kirkwood, R 1992, 'The control of bracken', Critical Reviews in Biotechnology vol. 12, pp 299-333.

Chavez Galarza, J, Pais, C & Sampaio, P 2010, 'Microsatellite typing identifies the major clades of the human pathogen *Candida albicans*', Infect Genet Evol vol. 10, pp 697-702.

Chen, XQ, Wang, JX, Tian, HL, Zhang, XC, Wen, J, & Zhou, SL 2008, 'Development of microsatellite markers for the bracken fern, *Pteridium aquilinum*', Molecular Ecology Resources vol. 8, pp 1491-1493.

Corander, J, Marttinen, P, Siren, J, & Tang, J 2008, 'Enhanced bayesian modelling in baps software for learning genetic structures of populations'. BMC Bioinformatics vol. 9, doi:10.1186/1471-2105-9-539.

Corander, J, Waldmann, P, & Sillanpaa, MJ 2003, 'Bayesian analysis of genetic differentiation between populations', Genetics vol. 163, pp 367-374.

Corander, J, Marttinen, P, Sirén, J, & Tang, J 2009, *BAPS: Bayesian analysis of population structure*, Manual version 5.3, available at: http://web.abo.fi/fak/mnf//mate/jc/software/baps.html

Der, JP, Thomson, JA, Stratford, JK, & Wolf, PG 2009, 'Global chloroplast phylogeny and biogeography of bracken (*Pteridium*; Dennstaedtiaceae)', American Journal of Botany vol. 96, pp 1041-1049.

Dighe, AS, Jangid, K, Gonzalez, JM, Pidiyar, VJ, Patole, MS, Ranade, DR, & Shouche, YS 2004, 'Comparison of 16S rRNA gene sequences of genus *Methanobrevibacter*', BMC Microbiology vol. 4, doi:10.1186/1471-2180-4-20.

Erre, P, Chessa, I, Munoz-Diez, C, Belaj, A, Rallo, L, & Trujillo, I 2010, 'Genetic diversity and relationships between wild and cultivated olives (*Olea europaea* L.) in sardinia as assessed by SSR markers', Genetic Resources and Crop Evolution vol. 57, pp 41-54.

Felsenstein, J 2006, *PHYLIP (Phylogeny Inference Package) Version 3.69*, Zoology Department, University of Washington, Seattle, Washington.

GenBank, http://www.ncbi.nlm.nih.gov/Genbank/ [accession numbers FJ177141, FJ177142, FJ177144, FJ177140, FJ177138, FJ177139, FJ177143]; accessed April 15, 2010).

Göttlicher, D, Obregón, A, Homeier, J, Rollenbeck, R, & Bendix, J 2009, 'Land-cover classification in the Andes of Southern Ecuador using Landsat ETM+ data as a basis for SVAT modelling', International Journal of Remote Sensing vol. 30, pp 1867-1886.

Hardy, OJ, Vanderhoeven, S, De Loose, M, & Meerts, P 2000, 'Ecological, morphological and allozymic differentiation between diploid and tetraploid knapwees (Centaurea jacea) from a contact zone in the Belgian Ardennes', New Phytologist vol. 146, pp 281-290.

Harper, JL 1982, *Population Biology of Plants*, Academic Press London, New York, San Francisco, pp. 729.

Hartig, K & Beck, E 2003, 'The bracken fern (*Pteridium arachnoideum*) dilemma in the Andes of South Ecuador', Ecotropia vol. 9, pp 3-13.

Hirai, M, Yoshimura, S, Ohsako, T, & Kubo, N 2010, 'Genetic diversity and phylogenetic relationships of the endangered species *Vaccinium sieboldii* and *Vaccinium ciliatum* (Ericaceae)', Plant Systematics and Evolution vol. 287, pp 75-84.

Korpelainen, H 1995, 'Mating system and distribution of enzyme genetic variation in bracken (*Pteridium aquilinum*)', Canadian Journal of Botany vol. 73, pp 1611-1617.

Marrs, RH, Johnson, SW, & Le Duc, MG 1998, 'Control of bracken and restoration of heathland. VI. The response of bracken fronds to 18 years of continued bracken control or 6 years of control followed by recovery', Journal of Applied Ecology vol. 35, pp 479-490.

Marrs, RH & Watt, AS 2006, 'Biological flora of the British Isles: *Pteridium aquilinum* (L.) Kuhn', Journal of Ecology vol. 94, pp 1272-1321.

Müller-Starck, G, Konnert, M, & von Wuehlisch, G 2001, *Labormanual zur Durchführung von Isoenzymanalysen bei der Buche (*Fagus sylvatica *L.)*, Bund-Länder-Arbeitsgruppe "Erhaltung forstlicher Genressourcen", Unterarbeitsgruppe "Biochemisch-genetische Analyse" Version 1.01.

Nei, M 1987, *Molecular Evolutionary Genetics*, Columbia University Press, New York.

Page, CN 1976, 'Taxonomy and phytogeography of bracken - a review', Botanical Journal of the Linnean Society vol. 73, pp 1-34.

Page, CN 2002, 'Ecological strategies in fern evolution: a neopteridological overview', Review of Palaeobotany and Palynology vol. 119, pp 1-33.

Page, RDM 1996, 'TreeView: An application to display phylogenetic trees on personal computers', Computer Applications in the Bioscience vol. 12, pp 357-358.

Parks, JC & Werth, CR 1993, 'A study of spatial deatures of clones in a population of bracken fern, *Pteridium aquilinum* (Dennstaedtiaceae)', American Journal of Botany vol. 80, pp 537-544.

Pegueroles, C, Arauz, P, Pascual, M, & Mestres, F 2010, 'A recombination survey using microsatellites: the o chromosome of *Drosophila subobscura*', Genetica vol. 138, pp 795-804.

Sheffield, E, Wolf, PG, & Haufler, CH 1989, 'How big is a bracken plant', Weed Research vol. 29, pp 455-460.

Soltis, DE, Haufler, CH, Darrow, DC, & Gastony, GJ 1983, 'Starch gel electrophoresis of ferns: A compilation of grinding buffers, gel and electrode buffers, and staining schedules', American Fern Journal vol. 73, pp 9-27.

Soltis, PS & Soltis, DE 2000, 'The role of genetic and genomic attributes in the success of polyploids', National Academy of Sciences Colloquium vol. 97, pp 7051-7057.

Speer, WD 1999, 'A systematic assessment of British and North American *Pteridium* using cpDNA gene sequences', Taylor, JA & Smith, RT (eds.) *Bracken Fern: Toxicity, Biology and Control*. Aberystwyth: International Bracken Group, pp. 37-42.

Speer, WD 2008, 'Phylogenetic and biogeographic relationships among North American and Hawaiian *Pteridium aquilinum* (L.) Kuhn (Dennstaedtiaceae) based on chloroplast *rps4* and *rps4-trnS* intergenic spacer sequences', American Fern Journal vol. 98, pp 179-193.

Speer, WD, Werth, CR, & Hilu, KW 1999, 'Relationships between two infraspecific taxa of *Pteridium aquilinum* (Dennstaedtiaceae). II. Isozyme evidence', Systematic Botany vol. 23, pp 313-325.

Thomson, JA 2000, 'Morphological and genomic diversity in the genus *Pteridium* (Dennstaedtiaceae)', Annals of Botany vol. 85, pp 77-99.

Thomson, JA 2008, 'Morphotype and conflicting taxonomies in *Pteridium* (Dennstaedtiaceae: Pteridophyta)', Fern Gazette vol. 18, pp 101-109.

Thomson, JA & Alonso-Amelot, ME 2002, 'Clarification of the taxonomic status and relationships of *Pteridium caudatum* (Dennstaedtiaceae) in Central and South America', Botanical Journal of the Linnean Society vol. 140, pp 237-248.

Tomiuk, J, Guldbrandtsen, B, & Loeschcke, V 2009, 'Genetic similarity of polyploids: A new version of the computer program POPDIST (version 1.2.0) considers intraspecific genetic differentiation', Molecular Ecology Resources vol. 9, pp 1364-1368.

Tomiuk, J & Loeschcke, V 1991, 'A new measure of genetic identity between populations of sexual and asexual species', Evolution vol. 45, pp 1685-1694.

Tryon, RM 1941, 'A revision of the genus *Pteridium*', Rhodora vol. 43, pp 1-31 and 37-67.

Tryon, RM & Tryon, A 1982, *Ferns and allied plants - with special reference in Tropical America*, Springer Verlag.

Van Puyvelde, K, Van Geert, A, & Triest, L 2010, 'ATETRA, a new software program to analyse tetraploid microsatellite data: comparison with TETRA and TETRASAT', Molecular Ecology Resources vol. 10, pp 331-334.

Werth, CR 1985, 'Implementing an isozyme laboratory at a field station', Varginia Journal of Science vol. 36, pp 53-76.

Wolf, PG, Haufler, CH, & Sheffield, E 1988, 'Electrophoretic variation and mating system of the clonal weed *Pteridium aquilinum* (L. Kuhn) (bracken)', Evolution vol. 42, pp 1350-1355.

SUPPORTING INFORMATION

Tab. 3-S1: Plate layout and instrument settings for DNA purification via BindIT 3.1 KingFisher software. After step 7, eluted DNA was transferred to plastic cups and kept at -20 °C.

Settings		Step 1 Bind MC2	Step 2 Wash MC3	Step 3 Wash MC4	Step 4 Wash Ethanol	Step 5 Wash MC5	Step 6 Elution MC6	Step 7 Disposal of magnetic beads
	Plate	A	B	C	D	E	F	G
	Layout	92 µl supernatant 8 µl magnetic beads 100 µl buffer	200 µl buffer	200 µl buffer	200 µl 80% Ethanol	200 µl buffer	20 µl buffer	
Beginning	Precollect	no	no	no	no	no	no	
	Release time [mm:ss]	no release	00:30	00:30	00:30	no release	00:15	00:20
	Release speed	-	fast	fast	fast	-	fast	fast
Mixing/ pause	Pause for manual handling	no	no	no	no	no	no	
	Mixing time [mm:ss]	05:00	01:00	01:00	01:00	01:00	10:00	
	Mixing speed	medium	fast	fast	fast	medium	medium	
End	Postmix	no	no	no	no	no	no	
	Collect count	3	3	3	3	3	6	
	Collect time [s]	1.5	1.5	1.5	1.5	1.5	1.5	

Tab. 3-S2: Morphological classification and allozyme banding pattern interpretation of 50 samples. The allozymes of each isozyme locus are numbered continuously beginning with the fastest (most anodal). a: *P. arachnoideum*, c: *P. caudatum*, ?: species classification was not possible

Sample ID	*Species classification based on morphology*	*Allozyme loci*																													
		MNR-B				*EST-A*				*EST-B*				*PGI-A*				*MDH-B*				*TPI-B*				*IDH*			
A10	c	1	1			1	2			1	2			2	2	2	5	1	3			2	2			1	3		
A11	a	1	1			2	2			1	1			4	5			3	3			2	2			1	1		
A12	a	1	1			2	2			1	1			4	5			3	3			1	2			1	1		
A5	a	1	1							1	2			2	4			3	3			2	2			1	1		
A6	a	1	1			2	2			1	2			2	4			3	3			2	2			1	1		
A7	a	1	1			2	2			1	1			5	5			3	3			2	2			1	1	1	3
A8	c	1	2			2	2			1	2			2	2	4	5	1	3	3	3	2	2			1	1		
A9	a	1	1			2	2			1	1			2	5			3	3			2	2			1	1		
B10	?	1	2							1	1	1	3	2	2	2	3	3	3			2	2			1	3		
B2	a	1	1			2	2			1	1			2	5			3	3			1	2			1	1		
B4	a	1	1			1	2			1	1			2	4			3	3			2	2			1	1		
B5	?	1	2			2	2			1	1			2	2	2	5	1	3	3	3	2	2			1	3		
B6	?	1	2			2	2			1	2	2	3	2	2	2	5	1	3	3	3	2	2			1	1	1	3
B7	c	1	2			2	2			1	1			2	2	2	4	1	3	3	3	2	2			2	2	2	3
B8	c	1	2							1	1	1	3	2	2	2	4	1	3			2	2			1	3		
B9	c	1	2			2	2			1	2	2	3	2	2			1	3			2	2			1	3	3	3
C1	c	1	2			2	2			1	2	3	3	2	2			1	1	1	3	2	2			1	3		
C10	c	1	2			1	2			1	1	2	3	2	3			1	3	3	3	2	2			1	3		
C11	a	1	1			2	2			1	1			2	5			3	3			2	2			1	1		
C2	a	1	1			1	2			1	1			3	4			3	3			2	2			1	1		
C3	a	1	1			1	2			1	1			3	3			3	3			2	2			1	1		
C4	c	1	2			2	2			1	2	2	3	2	4			1	3	3	3	2	2			1	1	1	3
C5	c	1	2							1	1	1	2	2	2			1	1	1	3	2	2			1	3		
C6	a	1	1			2	2			1	2			3	5			3	3			2	2			1	1	1	3
C7	?	1	1			2	2			1	1			4	5			1	3			2	2			1	1		

C6	a	1	1			2	2			1	1			2	2			3	3			3	3			2	2			3
C7	?	1	1			2	2			1	1			2	2			1	3			1	3			2	2			1
C8	?	1	1			2				2	3			3	3			2	5			3	3			2				3
C9	a	1	1			2	2			1	1			2	2			3	3			3	3			2	2			3
D1	a	1	1			2	2			1	1			2	2			3	3			3	3			2	2			3
D10	?	1	2			2	2			1	2			3	3			1	3			1	3			2	2			3
D5	a	1	1			2	2			1	1			2	2			3	3			3	3			2	2			3
D7	c	1	2			1	2			1	1			2	2			1	3			1	3			2	2			3
D9	a	1	1			2	2			1	1			3	3			3	3			3	3			2	2			3
E12	?	1	2			2				1	1			2	2			1	3			1	3			2	2			3
E2	a	1	1			2	2			1	1			2	2			3	3			3	3			2	2			3
E3	a	1	1			2	2			1	1			2	2			2	3			2	3			2	2			3
E4	a	1	1			2	2			1	1			2	2			3	3			3	3			2	2			3
E5	a	1	1			2	2			1	1			2	2			3	3			3	3			2	2			3
E7	a	1	1			2	2			1	1			2	2			2	3			2	3			2	2			3
F1	a	1	1			1	2			1	1			2	1			3	3			3	3			2	2			3
F10	a	1	1			2	2			1	1			2	2			3	3			3	3			2	2			3
F2	?	1	2			2	2			1	2			2	2			1	3			1	3			2	2			3
F3	a	1	1			2	2			1	1			2	2			2	3			2	3			2	2			3
F4	a	1	1			2	2			1	1			2	2			3	3			2	3			2	2			3
F8	c	1	2			2				1	2	3		3	3			2	5			3	3			2	2			3
G1	a	1	1			2	2			1	1			2	2			3	3			3	3			2	2			3
G2	c	1	2			2	2			1	1	2		3	3			1	3			1	1	3		2	2			3
G8	c	1	2			2	2			1	1	2		3	3			1	3			1	3			2	2			3
H1	c	1	2			2	2			1	2	2		3	3			1	3			1	3			2	2			3
H2	a	1	1			2	2			1	2			3	3			3	3			3	3			2	2			3
H7	a	1	1			2	2			1	1			2	2			3	3			3	3			2	2			3

Tab. 3-S3: Results of the rps4-trnS sequencing (2 different sequences) and interpretation of microsatellite banding pattern. Occuring microsatellite alleles were numbered continously beginning with the smallest.

Sample ID	*rps4-trnS sequence*	*Microsatellite loci*																							
		Pter03					*Pter04*					*Pter06*					*Pter09*					*Pter12*			
A10	-	1	6	13	14		2	3	9			6					6	24				5			
A11	-	1	6				6	7				1	3	6			3	31				1			
A12	-	1	4				3					2	5	10			9	12				1			
A5	-	24					7	8				3					11					1	5		
A6	2	1	3				8					2	6				17	29				1			
A7	-	1	2				3					6					9	16				1			
A8	2	1	6	13	14		2	9	14			6	7				6	9	13	18		1	2		
A9	2	1	7				7	8				6					10	35				1			
B10	2	1	6	15			2	5	7			6					6	8	13			1	5		
B2	-	1	2				3	7				6	8				10	30				1			
B4	2	1					7	8				3	6				6	7				1			
B5	-	1	6	14	17		2	5	10			3	6				6	18	24	32		1	2		
B6	2	1	12	13	14		2	7	14			2	4				11	21				1	2		
B7	-	1	6	14			2	3	11			1	6				6	18				5			
B8	-	1	6	14			2	5	10			5					15	22				5			
B9	-	1	6	13	15		1	2	5	10		5	6				6	16				5			
C1	-	1	6	14	17		1	2	7			4					6	11	18	24		5			
C10	1	1	6	13	15		2	14				6					6	11	20			5			
C11	-	24					6	8				7					31								
C2	2	1	19				9					1	6				3	12				1			
C3	-	1					7	8				3					2	12				1			
C4	-	1	6	14	18		2	7	11			6					6	8	15	19		1	2		
C5	-	1	14				2	11				5	7				6	10	16	24		5			
C6	-	1					7	8				2	6				10	28				1			

| | | |

Tab. 3-S4: Allele occurence (and frequencies) of five microsatellite loci for *P. arachnoideum* (*a*) with n = 30 and *P. caudatum* (*c*) with n = 19. Allele size "0" indicates null alleles. For one individual a present allele was only once counted indepently of zygosity or ploidy level.

Locus	*Allele size (bp)*	*a*	*c*
Pter03	174	25 (0.83)	18 (0.95)
	180	3 (0.1)	0
	186	1 (0.03)	0
	198	1 (0.03)	0
	226	5 (0.17)	16 (0.84)
	228	1 (0.03)	0
	240	7 (0.23)	0
	244	0	1 (0.05)
	252	0	8 (0.42)
	256	0	16 (0.84)
	258	0	5 (0.26)
	260	1 (0.03)	0
	262	0	4 (0.21)
	264	0	2 (0.11)
	266	1 (0.03)	0
	272	1 (0.03)	0
	0	4 (0.13)	0
Pter04	213	0	6 (0.32)
	217	0	1
	223	6 (0.2)	2 (0.11)
	225	0	2 (0.11)
	229	0	8 (0.42)
	231	6 (0.2)	2 (0.11)
	233	20 (0.67)	4 (0.21)
	235	11 (0.37)	2 (0.11)
	237	1 (0.03)	2 (0.11)
	239	0	3 (0.16)
	243	0	4 (0.21)
	251	0	4 (0.21)
	257	0	3 (0.16)
Pter06	196	2 (0.07)	(3) 0.16
	198	8 (0.27)	1 (0.05)
	200	10 (0.33)	1 (0.05)
	202	2 (0.07)	4 (0.21)
	204	2 (0.07)	3 (0.16)
	206	23 (0.77)	12 (0.63)
	208	3 (0.1)	5 (0.26)
	210	3 (0.1)	0
	214	1 (0.03)	0
	216	1 (0.03)	0

Pter09	260	2 (0.07)	0
	266	4 (0.13)	0
	268	0	1 (0.05)
	272	7 (0.23)	15 (0.79)
	274	1 (0.03)	0
	276	0	2 (0.11)
	278	4 (0.13)	1 (0.05)
	280	7 (0.23)	1 (0.05)
	282	4 (0.13)	5 (0.26)
	284	6 (0.2)	3 (0.16)
	286	0	2 (0.11)
	288	1 (0.03)	0
	290	3 (0.1)	4 (0.21)
	292	1 (0.03)	3 (0.16)
	294	1 (0.03)	1 (0.05)
	296	0	4 (0.21)
	298	1 (0.03)	2 (0.11)
	300	0	3 (0.16)
	302	0	1 (0.05)
	304	1 (0.03)	2 (0.11)
	306	0	1 (0.05)
	308	1 (0.03)	7 (0.37)
	310	1 (0.03)	0
	318	2 (0.07)	0
	320	3 (0.1)	0
	322	1 (0.03)	0
	324	1 (0.03)	0
	326	2 (0.07)	0
	328	0	1 (0.05)
	334	1 (0.03)	0
	340	1 (0.03)	0
Pter12	0	2 (0.07)	0
	360	28 (0.93)	8 (0.42)
	366	0	7 (0.37)
	368	1 (0.03)	0
	370	1 (0.03)	1 (0.05)
	374	1 (0.03)	12 (0.63)

CHAPTER 4

Forest clearing by slash and burn

Forest clearing by slash and burn

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1 INTRODUCTION

Burning is still the common method used by settlers to clear the primary forest for new farming areas. Every fire, irrespective of being lit inside or at the edge of the forest, kills the trees by burning or by the emerging heat. A fringe of dead, but not charred, trees is always found where forest has been cleared by fire. Later, when these heat-killed trees have completely dried up, they can be used to start a new fire.

2 VEGETATION SUCCESSION AFTER REPEATED BURNING

The remnants of previous burnings – charred trunks and large branches – are frequently left where they have fallen, because of the enormous efforts to remove them from the steep and often remote areas. Many areas have thus to be burned repeatedly until the spaces between the remaining logs are wide enough to plant beans or maize, or the pasture grasses *Setaria sphacelata* (Schumach.) Stapf & C.E.Hubb. ex Chipp. and *Melinis minutiflora* P. Beauv. At just 3–4 weeks after the fire, bracken fern (*Pteridium arachnoideum* (Kaulf.) Maxon) was observed, sprouting vigorously on both the burnt and the heat-killed areas, while it was absent in the intact primary forest. This de novo colonization by bracken may result via its readily germinating spores (Conway, 1953; 1957; Mitchell, 1973) or from already present leaf-producing lateral branches of elongating main rhizomes (Watt, 1940; Daniels, 1985) which form a dense network (Fig. 4-1) in the soil. Elongation growth and leaf sprouting is significantly stimulated by heat shock up to 70 °C (Roos & Beck, unpublished data).

Fig. 4-1: In situ “network” of bracken rhizomes in an area of 1×1 m from a depth of 0 cm to about 80 cm on an abandoned pasture within the RBSF.

Bracken and crops develop simultaneously after burning. Sooner or later the crops are replaced by tillering pasture grasses, in particular *Setaria sphacelata*, and the bracken fronds protrude mainly from spaces between the tussocks. When planted manually, *Setaria* grows faster than bracken and forms homogeneous pastures. However, since only the very young leaf blades and the tips of mature leaves are eaten by cattle, the carrying capacity of these pastures is low. The same is true for another grass, the stoloniferous, curtain-forming *Melinis minutiflora*, which maintains only one to three green leaves on a shoot. Bracken is not eaten by cattle due to its toxicity (Evans, 1986; Hannam, 1986; Fenwick, 1989). Therefore sooner or later the fern overtops the grasses and, by shading, weakens their growth. On flat slopes, its mainly horizontal fronds produce a closed canopy, preventing the establishment of a shade-intolerant vegetation beneath; but on the steep slopes its canopy is more open. Wind-dispersed seeds and light can protrude to the soil surface and a variety of herbaceous and shrubby plants are found in addition to

Pteridium in abandoned pasturelands. In particular, Asteraceae (*Baccharis latifolia*, *Ageratina dendroides*) and Melastomataceae (*Monochaetum lineatum*, *Tibouchina laxa*) can successfully compete with the fern (Stuart, 1988). For pasture rejuvenation and weed killing, farmers set fire whenever the weather permits. Especially the bushy Asteraceae survive recurrent fires and resprout from their base simultaneously with the emergence of the new fern fronds. A patchy vegetation results in which islands of bracken are separated by the 2–3 m high bushes and in which sporadic tufts of *Setaria* bear witness to the former pasture land. This is a highly stable type of vegetation (Fig. 4-2), due to the high propagation potential of the bushes via seeds and of the fern via rhizomes. It is encountered in many abandoned farming areas and thus may be addressed as a long-lasting serial stage if not a climax. The described successional sequence of stages has been documented phytosociologically by Hartig & Beck (2003). Many measures have been implemented to control bracken in agricultural areas but, due to the vigor of the rhizome system, none has been sustainable or successful (Lowday, 1986; Marrs *et al.*, 2000). Thus bracken is considered one of the world's most powerful weeds (Webster & Steeves, 1958) as it destroys arable land that has been managed by fire everywhere from the tropics to the temperate zones. Tree species characteristic of the former primary forests are very rarely found in the bracken–bush vegetation of abandoned pastures, and therefore a fast regeneration of a forest is very unlikely. The regenerative pressure of bushes, which produce immense amounts of wind-dispersed seeds, by far outstrips that of forest trees.

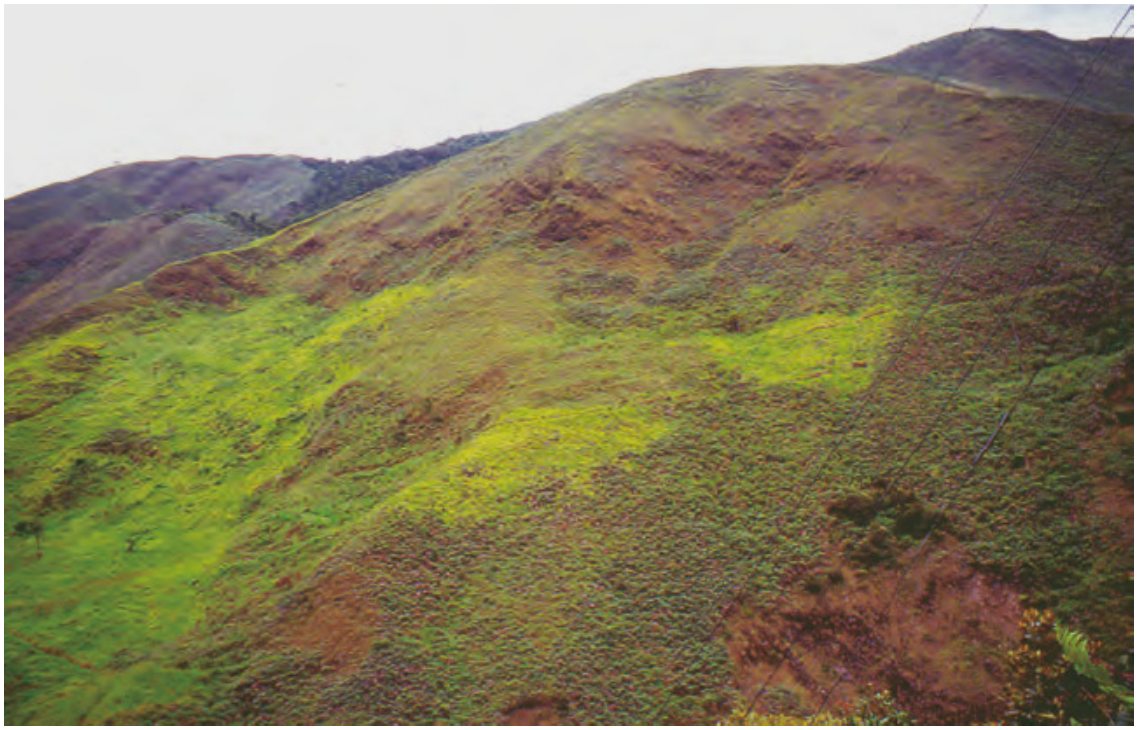

Fig. 4-2: Pastures (*bright green*) and bracken-dominated former pasture areas (*dark green to brownish*) which have been abandoned. At the crest of the mountain, remnants of the original forest can be detected.

Seeds and fruits of the latter are dispersed predominantly by birds and bats (Matt, 2001) and a single seed that is dropped by an animal on that kind of bushland has hardly any chance of germination. In addition, a substantial seed input from forest is unlikely as the remnants of the primary forests are usually far away.

3 CONCLUSIONS

As elsewhere in the tropics, farmers in the Andes of South Ecuador make extensive use of fire to convert primary forest into farming land and to foster their pastures. Repeated burning of the pastureland weakens the competitive strength of the pasture grasses, but increases the competitive strength of the extremely aggressive and fire-tolerant bracken fern. Pastures are finally abandoned when bracken becomes completely dominant. On steep slopes wind-dispersed seeds of several weeds germinate in the shade of the bracken leaves. A long-lasting successional vegetation

composed of dense patches of bracken interspersed with individual bushes develops. Since in these areas natural regeneration of the indigenous forest is very unlikely, reforestation may be the only way out of the dilemma caused by the extensive use of fire.

REFERENCES

Conway E (1953) Spore and sporeling survival in bracken (*Pteridium aquilinum* (L.) Kuhn). J Ecol 41, 289-294.

Conway E (1957) Spore production in bracken (*Pteridium aquilinum* (L.) Kuhn). J Ecol 45, 273-284.

Daniels R (1985) Studies in the growth of *Pteridium aquilinum* (L.) Kuhn (bracken). 1. Regeneration of rhizome segments. Weed Res 25, 381-388.

Evans WC (1986) The acute diseases caused by bracken in animals. In: Smith RT, Taylor JA (eds) Bracken: Ecology, Land Use and Control Technology, 1985. Parthenon, Leeds, pp 121-132.

Fenwick GR (1989) Bracken (*Pteridium aquilinum*) – toxic effects and toxic contituents. J Sci Food Agric 46, 147-173.

Hannam DAR (1986) Bracken poisoning in farm animals with special reference to the North York Moors. In: Smith RT, Taylor JA (eds) Bracken: Ecology, Land Use and Control Technology, 1985. Parthenon, Leeds, pp 133-138.

Hartig K & Beck E (2003) The bracken fern (*Pteridium arachnoideum*) dilemma in the Andes of South Ecuador. Ecotropia 9, 3-13.

Lowday J (1987) The effects of cutting and asulam on numbers of frond buds and biomas of fronds and rhizomes of bracken *Pteridium aquilinum*. Ann Appl Biol 110, 175-184.

Matt F (2001) Pflanzenbesuchende Fledermäuse im tropischen Bergregenwald: Diversität, Einnischung und Gildenstruktur – Eine Untersuchung der Fledermausgemeinschaften in drei Höhenstufen der Andenostabdachung des Pdocarpus Nationalparks in Südecuador. PhD Thesis, University of Nürnberg-Erlangen. Erlangen.

Marrs RH, Johnson SW, Le Duc MG (2000) Control of bracken and restoration of heathland. VI. The response of bracken fronds to 18 years of continued bracken control or 6 years of control followed by recovery. J Appl Ecol 35: 479-490.

Mitchell J (1973) Mobilization of phosphorus by *Pteridium aquilinum*. Plant and Soil 38, 489-491.

Stewart GH (1988) The influence of canopy cover on understory development in forests of the Western Cascade Range, Oregon, USA. Vegetatio 76, 79-88.

Watt A (1940) Contributions to the ecology of bracken (*Pteridium aquilinum*).I. The rhizome. New Phytol 39, 401-422. 1940.

Webster B & Steeves T (1958) Morphogenesis in *Pteridium aquilinum* (L.) Kuhn. General morphology and growth habit. Phytomorphol 8, 30-41.

CHAPTER 5

The invasive capacity of bracken

Growth of tropical bracken (*Pteridium arachnoideum)*: Response to weather variations and burning

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ABSTRACT

The ecology of southern bracken, which occurs in tropical regions, is not well known. We studied its response to weather variations and burning in the South Ecuadorian Andes, where this weed had already overgrown 40% of the pastureland. In field observations a constant one to one ratio of emerging and dying leaves suggested limitation of frond density by nutrient shortage. Short term deviations from that ratio could be related to weather variations. Spells of dry weather temporarily increased mortality but stimulated emergence of new fronds. Life-span of the fronds produced immediately after a fire was longer than of those produced during unaffected bracken growth. A burst of frond development during the initial two to three months was observed after a fire followed by self-thinning to a stable level. To analyse the effect of fire on bracken, rhizomes were treated with heat pulses. Rhizomes were heat tolerant up to 70°C and frond production from short shoots was enhanced by elevated temperature. Burning apparently releases apical dominance of developed fronds, as does cutting, and stimulates bud break. The local practice of pasture maintenance in Ecuador of repeated burning favors growth of the fern.

Nomenclature: bracken, *Pteridium aquilinum* agg.; southern/tropical bracken (bracken of the southern hemisphere), e.g. neotropical *Pteridium arachnoideum* (KAULF.) MAXON and *Pteridium caudatum* (L.) MAXON (nomenclature based on Der *et al.*, 2009)

Keywords: southern bracken, invasive ferns, vegetation dynamics, fire ecology, heat effects on rhizome.

INTRODUCTION

Bracken (*Pteridium* spp.) is one of the most agressive weeds worldwide, infesting pastures, forested areas, and bushland when it causes severe problems for farmers, foresters, and conservationists (Pakeman *et al.*, 1994). Due to its variable and robust rhizome system, it resists any kind of mechanical, herbicidal (Marrs *et al.*, 1998; Le Duc *et al.*, 2003), or biological control (Burge & Kirkwood, 1992; Burge *et al.*, 1986). It propagates readily by spores and also by long-living rhizomes, is highly resistant against pathogenic microorganisms, and is poorly palatable for grazing animals. Its, allelopathic effects on other plant species, tolerance of weather extremes and bush-fires, and its remarkable genetic plasticity strengthen its competitiveness as a weed (Page, 1986). Bracken (*Pteridium aquilinum* (L.) Kuhn) as a cosmopolitan plant is genetically not uniform and appears as an aggregate which by morphological and molecular characters can be differentiated into a northern and a southern group (Page, 1976; Der *et al.*, 2009). The northern group whose leaves are not frost resistant (the *"aquilinum"* complex) is ecophysiologically characterized by a life cycle that is synchronized by the winter break. The southern group [including the diploid *P. arachnoideum* (KAULF.) MAXON and the allotetraploid *P. caudatum* (L.) MAXON (Thomson, 2000; Der *et al.*, 2009)] comprises mainly tropical representatives with year-round growing. Uninterrupted growth of rhizomes and leaves results in tall plants, whose fronds can grow as high as four meters at the edge of a forest (K. Roos, unpublished). In contrast to the well defined ecological requirements of the northern bracken (Page, 1986; Evans *et al.*, 1990; Ader, 1990; Pakeman *et al.*, 2000), ecophysiology of the tropical bracken is less well known (Hollinger, 1987; Bray, 1991; Alonso-Amelot & Rodulfo-Baechler, 1996; Quitete-Portela *et al.*, 2009). Previous observations suggest that higher rainfall has a positive effect on growth whereas low temperature has a negative effect (Quitete-Portela *et al.*, 2009).

In the Andes of southern Ecuador vast areas of primary mountain forest have been cleared by slash and burn to produce pastures (Hartig & Beck, 2003; Beck *et al.*, 2008a). After burning all fallen trunks and branches, pasture grasses such as *Setaria sphacelata* (Schumach.) Stapf and C.E. Hubb. ex Chipp., and *Melinis*

minutiflora P. Beauv., are planted or sown. For the first years the grasses develop well, but gradually bracken takes over, prompting the farmers to set fire to the pastures, which damages the fern less than the grasses. Eventually, the pastures are abandoned and farmers clear another piece of the pristine forest. In the study region in the South Ecuadorian Andes, about 40% of the area where forest has been cleared consists of such bracken dominated sites (Göttlicher *et al.*, 2009).

There are numerous reports that fire kills the standing fronds but stimulates growth of bracken (Agee & Huff, 1987; Ingram, 1931; Sharik *et al.*, 1989). However, in an experiment by Flinn and Pringle (1983), rhizomes of northern bracken were not heat resistant. Bracken rhizomes consist of a fast growing main axis, termed long shoot, which usually does not produce fronds, and the frond producing lateral (short shoots) (Watt, 1940). Long shoots are found deeper in the soil than the laterals, which usually concentrate in the top soil and thus should be particularly endangered by fires. Intermediates of both rhizome types have also been found (Watt, 1940; Webster & Steeves, 1958).

In this study we report on growth dynamics of the neotropical bracken, *Pteridium arachnoideum*, as affected by weather variations and burning. Different weather variables were used to identify possible triggers of frond emergence and dying at an undisturbed bracken site in southern Ecuador. After burning, regrowth of bracken populations of varying density were studied and compared with the growth dynamics of the undisturbed bracken vegetation. The field studies were supplemented by laboratory experiments, to investigate the effects of heat pulses on the bracken rhizome. We investigated heat tolerance of long and short shoots separately and examined survival, growth, and frond production. In a field experiment frond emergence after burning was compared with that after cutting to examine whether frond emergence after fire results only from release of apical domincance of resting frond buds (Burge & Kirkwood, 1992) or, is stimulated by burning.

MATERIALS AND METHODS

The Study Site. The study area, located in the upper Rio San Francisco valley in the Andes of South Ecuador, extends over a horizontal distance of 1.2 km and vertically from 1,800 to 2,200 m above sea level. It borders the "Reserva Biológica San Francisco" (3°58'30'' S, 79°4'25'' W) which is part of the Podocarpus National Park. A comprehensive geographical description of the area has been presented by Beck *et al.* (2008b). The steep slopes harboring the research area are covered by open vegetation consisting of sporadic tussocks of pasture grass *Setaria sphacelata* under a more or less closed canopy of bracken fronds, up to two meters high, which is interspersed with scattered bushes. Suppression and displacement of the pasture grasses by bracken has been attributed to the common practice of pasture maintance by burning (Hartig & Beck, 2003; Beck *et al.*, 2008a). The study area was abandoned and had not been used as pasture for at least 11 years.

Long-term Field Observations. *Growth Of Bracken.* Within an area of 3.5 ha six circular plots with a diameter of two meters each (3.14 m^2) were randomly established. These are referred to as control plots. Each bracken frond in the plots was individually tagged and followed from emergence until death. From November 2005 until May 2008, the total numbers of fronds, new leaves, and dead leaves were recorded every four weeks. Additionally, the developmental states of the individual bracken fronds (unfolding, mature, senescent = browning of ≥ 50% of the frond area, and dead) were monitored on two of these plots. Thus, the total life-span and the length of the developmental phases of bracken fronds could be determined.

To assess the impact of weather variations on the growth of bracken, weather conditions were continuously recorded with an automated climate station[1] (Rollenbeck *et al.*, 2007) which was installed about one kilometer away from the study site (03°58'21" S, 79°04'35" W, altitude: 1,960 m above sea level).

Frond Regeneration After Fire. In October 2004 and December 2005 big bushfires devastated major parts of the study area. However, the area of control plots was not affected. Immediately after the fires, sites for monitoring regrowth of bracken were selected. After the fire in 2004 an area of 7.4 ha was selected for that purpose and after the fire in 2005 another area of 3.7 ha. Within the first site 13 plots and in the second 10 plots were established. Plot size and mode of monitoring were identical to the control plots. The first series of plots were investigated for 18 months and the second for 12 months.

Heat Penetration Study. To examine heat penetration into soil, a bracken-covered area (50 × 20 m) was burned two days after the last rain and soil temperatures were measured every five minutes at two and five centimeters depth, using PT1000 thermocouples[2] connected with an automated climate station[1].

Frond Regeneration After Cutting Versus Heating. To analyse whether frond emergence after fire results only from release of apical dominance of resting frond buds, or is additionally stimulated by burning, nine plots of one m^2 each with similar bracken frond densities (on average 12 fronds/m^2) were installed randomly in the field (in an area of 50 × 100 m). Simultaneously, one set of 3 plots was burned, while in another set (3 plots) bracken fronds were cut near the ground, and the remaining 3 plots were left untreated (controls). On all plots the emerging fronds were tagged and regeneration was observed.

Heatshock Treatment Of Bracken Rhizomes. To examine reactions of rhizomes with respect to temperature in more detail, a heat pulse experiment was conducted in which survival, extension growth and frond production were determined. Rhizomes (in total 469) were collected in the field and separated into long and short shoots (Watt, 1940). Rhizomes with a minimum length of 12 cm, [mean length 26.6 ± 0.4 cm (SE)] were used, and the numbers of buds on the individual rhizomes were determined before heat treatment. Most of the rhizomes had at least one bud, but also rhizomes without buds were tested to assess the importance of buds for survival. Applied temperatures were 40, 45, 50, 55, 60, 70 and 80°C. Heat treatments

were administered in two ways: i) by dipping the rhizomes for 2 min in a preheated water bath or ii) heating them together with original moist soil in a temperature-controlled oven to the desired temperatures at which they remained for 10 min. Warming-up of the soil (with rhizomes) took between 20 and 60 min depending on the final temperature. Heating in soil is closer to the situation in the field because it allows for the slow penetration of the heat wave in moist soil. After the exposure to the elevated temperatures the samples were cooled to ambient room temperature. Thereafter, all rhizomes were planted in original soil, and cultivated at near-natural temperature conditions under a plastic roof (to avoid soaking of the rhizome bed by the daily rains). The rhizomes were excavated after two months of cultivation and their condition, change in length and frond production were determined. Survival of rhizomes was assessed by their consistency and color. Healthy rhizomes have a durable coat and their interior is white while dead rhizomes are soft and brown. Control rhizomes were handled in the same manner, but without the heat treatment.

Data Analysis. The program SPSS 13.0 (SPSS, 2004) was used for the statistical analyses. Normality of data distribution was evaluated with the Shapiro-Wilk test. In the case of new emerging and dying fronds (at the control plots), length increment and frond production after heat shock, data were not normally distributed (even after different trials of data transformation) and therefore, direct relations were analysed with Mann-Whitney U-tests. A Spearman correlation was used to prove the coincidence of emerging and dying fronds at the untreated plots. A binary logistic regression was used to determine the effects of different variables on survival of rhizomes after the heat shock experiment.

With regard to the relation between weather and frond dynamics, the data for precipitation, air temperature, relative air humidity, and solar irradiance (original temporal resolution is 10 minutes) were aggregated to 4-weekly sums of rainfall and 4-weekly averages of solar radiation, air humidity and air temperature (encompassing average minimum and maximum air temperature) to match the 4-week period of bracken monitoring (Fig. 5-1). The Pearson correlation coefficient was used to analyse the relation between emergence and dying of bracken fronds and

weather parameters (Tab. 5-1). Because 4-week sums and average values will not show shorter periods of extreme climate stress that might affect bracken frond dynamics, additional analyses were carried out to detect such events. Three variables were selected:

i) The relative fraction of days per monitoring period with rainfall<0.1 mm ($fR_{<0.1}$)

$$fR_{<0.1} = \frac{\sum_{1}^{d} D_{<0.1}}{d} \quad [1]$$

where $fR_{<0.1}$ is the relative frequency of days with rainfall<0.1 mm, d the total number of days of the respective bracken frond monitoring period and $D_{<0.1}$ the number of days with rainfall<0.1 mm.

ii) The relative frequency of days with low average relative air humidity (<70%) per monitoring period ($frH_{<70}$)

$$frH_{<70} = \frac{\sum_{1}^{d} D_{<70}}{d} \quad [2]$$

where $frH_{<70}$ is the relative frequency of days with average relative air humidity<70%, d the total number of days of the monitoring period and $D_{<70}$ the number of days with average relative air humidity<70%.

iii) The relative frequency of days with high average daily irradiance (>190 W/ m^2) per monitoring period ($fQ_{>190}$)

$$fQ_{>190} = \frac{\sum_{1}^{d} D_{>190}}{d} \quad [3]$$

where $fQ_{>190}$ is the relative frequency of days with average solar irradiance>190 W/m^2, d the total number of days of the bracken monitoring period and $D_{>190}$ the number of days with average solar irradiance>190 W/m^2.

The three resulting frequency time series were correlated (Pearson correlation analysis) with the time series of new and dead frond abundance to investigate the impact of extreme weather situations on bracken frond dynamics.

RESULTS AND DISCUSSION

Undisturbed Growth Of Bracken. In contrast to the annual growth pattern of the northern bracken, which is interrupted and thus synchronized by the winter break, the tropical bracken shows continuous growth. The life-time of the individual fronds showed a median of 8 months (UQ = 10, LQ = 5, maximum 18 months, minimum<1month), which is longer than the frost-limited life-span of leaves of the northern bracken (approximately 6 months). Unfolding took less than one month, the length of the mature state was between three and seven months, and the phase of senescence lasted up to two months. In the course of the years 2005 to 2008 in the control plots on average 16% of the fronds were unfolding, 22% senescent while 62% were considered as mature. Frond density showed moderate fluctuations (Fig. 5-1) around an average value of 17.2 ± 0.7 (SE) fronds/m^2 during the observation period of 2.5 years. Frequently dying of old leaves coincided temporally with the emergence of new ones (r_s = 0.51, P < 0.001) and the average rates of both processes were not significantly different [2.0 ± 0.3 (SE) dying fronds/m^2/$months^1$, and 2.3 ± 0.2 (SE) developing fronds/m^2/$months^1$, P > 0.40]. This almost perfect one to one ratio suggests a physiological interrelation of both processes, given that both take place on the same set of rhizomes. A similar observation has been reported from pastures in New Zealand, which were infested by another species of southern bracken, *Pteridium esculentum* (Bray, 1991). The studies reported here were performed in an open bracken vegetation and the balance between dying and emerging leaves did not exhibit a noticeable seasonal variation. This close interrelation could be explained as resulting from inorganic nutrient shortage when the development of a new leaf requires nutrients remobilized from senescing leaves. Bracken generally prefers soils with a medium to high nutrient content (Ader, 1990; Waring & Major, 1964) but the topsoils of the study site are poor in macronutrients (Hamer *et al.*, 2009; Potthast *et al.*, 2010). Limitation of bracken growth by edaphic

factors is also indicated by the long lasting differences in the density of the bracken canopy on the investigated plots. An average and stable density between 15 and 20 fronds/m^2 (Fig. 5-1) constitutes a moderate level of bracken infestation while frond densities of 3 to 4 fronds/m^2 indicate less favorable conditions, such as compact or waterlogged or extremely nutrient-poor soils (Brown, 1986; Watt, 1976).

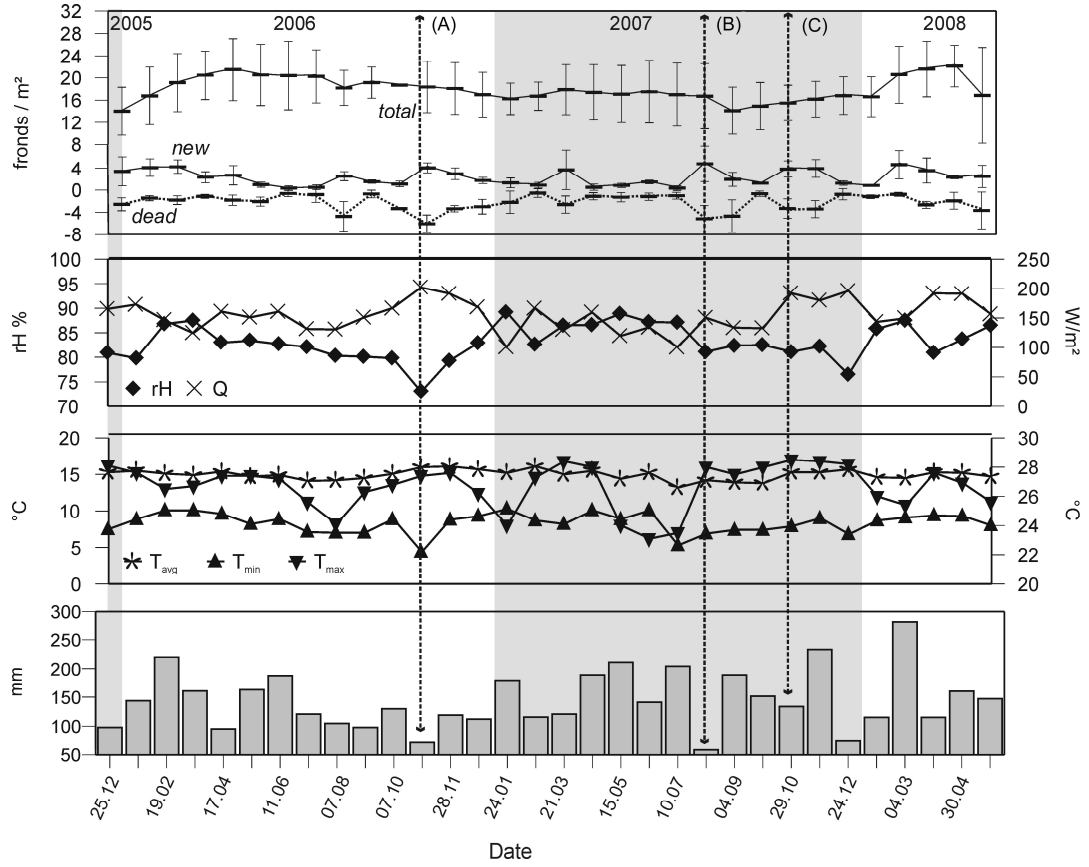

Fig. 5-1: Monthly bracken frond density (mean ± SE per m2 of six plots, observed in 4-week-intervals) and the 4-week-averages of relative humidity (rh), solar irradiance (Q), temperature (T average, minimum and maximum) and rainfall sums at the Estación Científica San Francisco meteorological station in the course of 2.5 years. Note that the dates of the abscissa represent the last day of each 4-week period. A, B and C (arrows) mark extreme weather situations.

Weather Effects On Growth Dynamics. In all plots bracken frond dynamics showed only short-term deviations from the mentioned on-to one-ratio that can be related to spells of weather extremes (Tab. 5-1). The emergence of new fronds correlates with periods of higher maximum air temperature, enhanced irradiance (daily average>190

W/m²), and periods of reduced air humidity. High irradiance and air temperatures also led to increased soil temperatures which confirms previous findings with northern bracken that frond emergence is triggered by higher soil temperatures (Pitman & Pitman, 1990) and that bracken growth is promoted by direct radiation (Bendix *et al.*, 2009).

Tab. 5-1: Correlations (*r* values) between weather elements and frond dynamics for the 4-week periods. T = air temperature, rH = relative air humidity, Q = solar irradiance, R = Rainfall. For other acronyms refer to equations 1-3. Significant *P*-values are given in bold.

	Emerging Fronds	***Dying Fronds***
T_{avg}	+0.24 (*P* = 0.18)	+0.07 (*P* = 0.70)
T_{max}	**+0.36 (*P* = 0.04)**	+0.17 (*P* = 0.35)
T_{min}	-0.02 (*P* = 0.91)	**-0.37 (*P* = 0.04)**
rH	-0.22 (*P* = 0.22)	**-0.43 (*P* = 0.01)**
$frH_{<70}$	**+0.42 (*P* = 0.02)**	**+0.42 (*P* = 0.02)**
Q	**+0.37 (*P* = 0.04)**	+0.26 (*P* = 0.15)
$fQ_{>190}$	**+0.52 (*P* = 0.002)**	+0.31 (*P* = 0.08)
R	-0.04 (*P* = 0.82)	-0.31 (*P* = 0.08)
$fR_{<0.1}$	+0.20 (*P* = 0.27)	+0.21 (*P* = 0.24)

The dieback of old fronds shows slightly different relations to the observed weather patterns (Tab. 5-1). We found significant negative correlations between the average air humidity and the number of dying fronds. The positive correlation to the spells of low air humidity extremes (r = +0.42 for days with humidity<70%) indicates that exceptionally dry periods led to enhanced frond mortality, which is in line with the findings by Roberts *et al.* (1980). The significant negative correlation between frond mortality and minimum temperatures can be interpreted as a side effect of extreme low humidity concomitant with low cloudiness and thus, nocturnal radiative cooling of

the air. This is confirmed by the highest correlation of meteorological parameters between relative air humidity and minimum temperature ($r = +0.57$, $P < 0.001$).

One reason for the relatively low correlations in Table 5-1 might be the relative low temporal resolution of the time series. Extraordinary weather events of shorter duration (few days) most likely affect frond emergence and mortality, but might be hidden in the 4-week averaging of the meteorological data. However, the following detailed analysis of short time periods demonstrates the modulation of bracken dynamics by short weather extremes. The best example is the dry period in October/November 2006 [Line (A) in Fig. 5-1]. This period was characterized by the lowest average humidity and the highest average irradiance, resulting in elevated air temperature during the day, but low nocturnal temperatures due to unhampered radiation emission. During this spell, relative humidity was below 60% for five consecutive days (and even below 50% for two days). Highest monthly frond mortality was observed during that period, concomitantly with an increased emergence of new fronds. Other periods of enhanced frond development were in August 2007, and in October/November 2007 [lines (B) and (C) in Fig. 5-1]. August 2007 was characterized by a slightly reduced average humidity on single days, while solar irradiance was on the level of the long-term average, except three consecutive clear days with an averaged radiation>200 W/m^2. The strikingly low precipitation combined with that spell of high radiation was paralleled by an enhanced frond mortality which in that case slightly exceeded frond emergence. The situation in October/November 2007 [line (C) in Fig. 5-1] was somewhat different from the above mentioned October 2006 [line (A)]. Precipitation and relative humidity were higher than one year before while solar radiation was similarly high. Under these conditions frond production slightly exceeded frond mortality.

In summary, days with solar radiation averaging>190 W/m² correlated best with frond emergence, while those of dry days (humidity<70%) matched frond mortality. This implies that high solar irradiance fostered leaf development and low air humidity enhanced frond mortality.

Regrowth Of Bracken After A Fire. In the pilot heat penetration study, heat pulses up to 40°C at two centimeters, and up to 30°C at five centimeters soil depth were recorded. At both soil depths the maximum temperatures were measured about 12 minutes after the arrival of the fire at the particular area, but cooling was much slower. At two centimeters depth the initial temperature of 16°C was approached after three hours while at five centimeters depth it took five hours. Leaf emergence from the burnt soil was apparently not stopped by that low intensity of heat; however young fronds emerging from the burnt soil during the first days after the fire did not survive the first month. A burst of frond production was recorded two months after the fire and, depending on the original density of the bracken rhizomes, a more or less closed bracken canopy formed (Fig. 5-2B and C, and Fig. 5-3). Maximum bracken frond density occured between five and seven months of regeneration.

Fig. 5-2: Dynamics of bracken after a fire. A, the day after the fire; B, one month later with some new emerging fronds (red arrows); C, closed canopy three month after the fire; D; large-scale dying of the first generation of fronds one year after fire.

Dynamics of regrowth was plotted separately for plots exhibiting different levels of infestation by bracken. On slightly infested areas a patchy bracken canopy formed at an average density of 3 fronds/m^2 and a maximum density up to 8 fronds/m^2. On such areas resprouting was observed from bunches of the former pasture grass that had survived the fire. On moderately infested areas the bracken canopy leveled off at an average density of 13 fronds/m^2 (Fig. 5-3A) passing through a maximum value of 24 fronds/m^2 at some plots (not shown in the Figure which presents average values and SE). A few small grass tufts were observed beneath fronds, and young shoots of fire resistant bushes like *Ageratina dendroides* and *Baccharis latifolia* sprouted from the scattered rootstocks. Such accompanying flora was not observed on heavily infested plots where an average of 28 (Fig. 5-3B) and a maximum of 41 fronds/m^2 were recorded (not shown in the Figure).

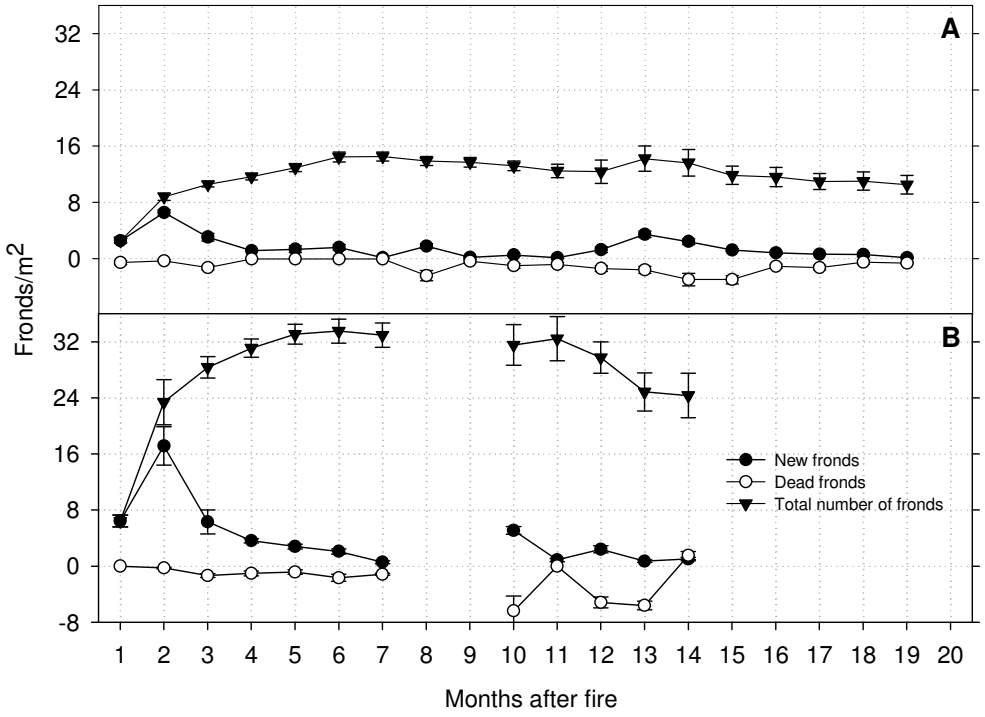

Fig. 5-3: Regrowth of bracken after the fires in October 2004 (A) and December 2005 (B), respectively, resulting in a moderate (A) and in a high density (B) of the canopy. Means ± SE per m2 of five plots each are shown in each graph.

Obviously, burning of the vegetation had a synchronizing effect on the development of bracken fronds. Mass dying of fronds was observed when their maximum life-time was reached (Fig. 5-2D). However, this effect did not result in a pronounced wave-like characteristic of the frond density, as new fronds immediately replaced the dead ones. Only a slight reduction of the leaf density was observed 10 to 11 months after the maximum rate of frond emergence (12 to 13 months after fire, Fig. 5-3), reflecting synchronous dying of the initially produced leaves. In the less and moderately infested plots a slightly elevated rate of leaf production followed that decrease. In the heavily infested plots a substantial dieback of leaves (around 17%, Fig. 5-3B) was recorded 12 to 13 months after the fire. On average, on the burnt plots, bracken frond cover declined by 23% during the following 14 months. Infraspecific competition resulting in self-thinning appears to be the cause for this reduction to a stable level. Northern bracken is known as a light demanding, shade intolerant pioneer species (Ingram, 1931; Stewart, 1988) and the southern bracken apparently is no exception to this. Overlapping of the fronds resulting in a steep

gradient of light intensity in the canopy layer may cause premature senescence of the shaded fronds.

Records of individually tagged fronds showed a significantly increased average life-time of those fronds produced immediately after the fire (nine months) compared to the fronds in the undisturbed plots (eight months) (n = 1174, Z = -6.18, $P < 0.001$). The increased life-span by one month supports the idea that low nutrient availability restricts the life-time of fronds in the undisturbed plots. In a perhumid climate (>10 humid months per year; Lauer & Bendix, 2004) nutrients are quickly allocated after a fire from the ash to the topsoil thus alleviating the nutrient shortage of the plant for a while. Beneficial effects of burning on northern bracken have also been reported (Page, 1982).

Most short rhizomes grow in the soil layer between two and nine centimeters depth (Beck *et al.*, 2008a), and were not damaged by the heat pulse from the fire which in the moist soil is very moderate. Stimulation of leaf-bud sprouting by the transient heat (30 to 40°C) as measured in the pilot burning experiment is conceivable.

Examination Of Heat Tolerance Of Bracken Rhizomes. To investigate the physiological effects of the moderate heat pulses on the southern bracken, rhizomes were separated into long and short shoots and subjected to heat treamtents at various elevated temperatures. In the work of Flinn & Pringle (1983) rhizomes were immersed in a preheated water bath. In nature, rhizomes of the southern bracken are commonly embedded in moist soil and therefore in the work presented here heat pulses were applied to the rhizomes in original soil (in an oven) and for comparison without soil in a preheated water bath.

Survival. Overall, dipping the rhizomes for two minutes in preheated water was less harmful than heating them in wet soil, for which, however, more time was required to attain the maximum test temperature. Some damage might have already occurred

during this warm-up phase. Statistical analysis of the results (Tab. 5-2) revealed that the temperature and the mode of heating, in addition to the type, diameter and length of rhizome were the important variables for the susceptibility and reactions of bracken rhizomes to a heat pulse. In contrast, the initial number of buds had no influence on the survival of heated rhizomes. Fewer than 25% of all rhizomes died in the course of the post-treatment cultivation period (Fig. 5-4). Rotting of the cut ends was observed frequently also on control rhizomes. Therefore, rhizomes which were only slightly rotted were counted as survivors. With respect to survival, long shoots were less resistant than short shoots, especially when they had been subjected to the heat pulse in soil. Under these conditions, few samples survived temperatures above 50°C, whereas most survived heat treatment in the water bath even at 80°C (Fig. 5-4). However, the short shoots survived 80°C irrespective of the mode of heat pulse and thus were significantly more heat resistant than the long shoots ($P < 0.001$). This was not unexpected, as the long shoots are commonly found deeper in the soil where heat waves from bushfires do not penetrate. A similar explanation was presented by Flinn & Pringle (1983) for an "(imprecise) correlation between depth of location of rhizomes and their tolerance to heat damage". As heat tolerance of the short rhizomes was remarkably high, they have a good chance for surviving the common bushfires which generate soil temperatures well below 100°C as measured in the pilot burning experiment.

Tab. 5-2: Influence of different variables on survival of rhizomes after heat pulse. Analyses were done with binary logistic regression with method of heat treatment and rhizome type as categorical variables, B gives the slope and W is the Wald value corresponding to P, significant P-values are given in bold.

Variables	***B***	***W***	***P***
Temperature	**-0.23**	**7.47**	**0.006**
Method of heat treatment: (1) water/ (0) oven	**1.95**	**37.17**	**<0.001**
Rhizome type: (1) short/ (0) long shoot	**1.36**	**13.72**	**<0.001**
Length of the rhizome	**0.06**	**6.41**	**0.011**
Diameter of the rhizome	**2.22**	**5.76**	**0.016**
Number of buds on the rhizome (incl. without buds)	0.24	2.69	0.101

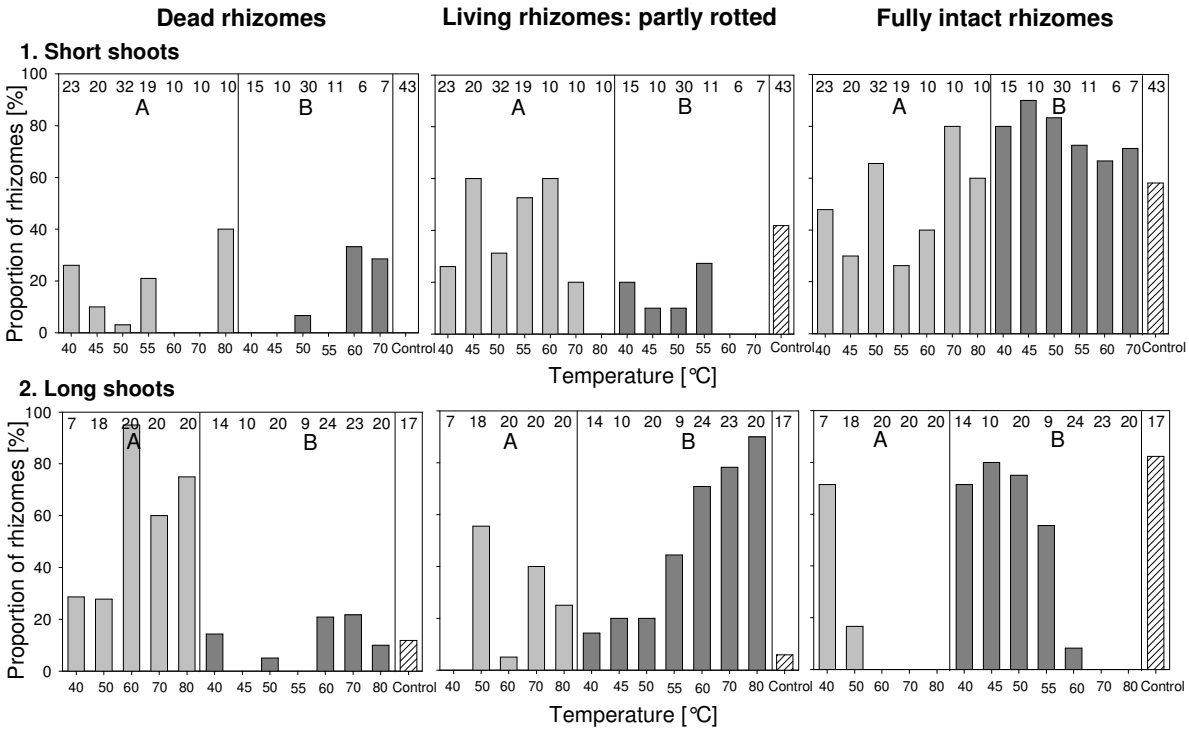

Fig. 5-4: Survival of bracken long and short shoots after heat treatments A) in soil, B) in water bath at various temperatures and subsequent cultivation for 2 months in soil under semi-natural environmental conditions. Rhizomes (short and long shoots) were differentiated into dead and living, and living rhizomes were further differentiated into partly rotted (if lenght after 2 months was shorter than before) and fully intact. The numbers in the top of the panels indicate the size of the samples subjected to the respective treatment.

Effect Of A Heat Pulse On Elongation Growth. With long shoots, heat pulses did not stimulate elongation growth. On the contrary, growth of the long shoots was significantly inhibited at most experimental temperatures, irrespective of how the heat pulse was applied (Fig. 5-5). Inhibition was mainly due to rotting, partly of the tissue damaged by the heat, partly by natural rotting of the cut rhizomes. Short shoots, however, showed a significant increment after the heat pulses in soil at 40 and 55°C, and at 50°C in the water bath (Fig. 5-5). Apparently the heat treatment in soil was more effective in stimulating growth.

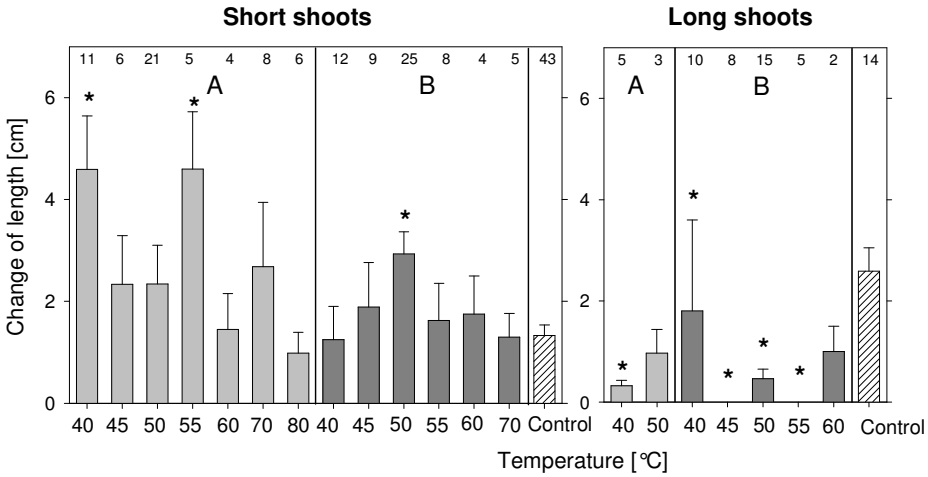

Fig. 5-5: Change of length of short and long shoots 8 weeks after heat treatment A) in soil, B) in water bath (means ± SE, significant differences between heat-treated rhizomes and controls were calculated by Mann-Whitney U-tests with all $P < 0.003$ and are marked with an asterisk). The numbers in the top of the panels indicate the actual numbers in the respective category. Only the fully intact rhizomes were counted.

Effect Of Heat Pulse On Frond Production. A significant stimulation of frond production from short rhizomes was observed by heat pulse temperatures between 40/45°C and 55°C (Fig. 5-6). The positive effect of the heat treatment was more pronounced when the rhizomes were heated in soil as compared to the wet heat pulse. Above 55 (wet heat pulse) and 60°C (in soil), fronds were not produced, although the rhizomes survived heating up to 80°C. In the study of Flinn & Pringle (1983) survival and resprouting after the heat treatment was investigated and although some of the rhizomes survived 55°C resprouting was consistently less than

in the control. This behavior was different from a burning experiment, in which bracken was the first of six rhizomatous species to resprout after the fire (Flinn & Wein, 1988). In the experiment reported here short shoots of southern bracken did not only survive temperatures above 55°C but could react to a transitory heat pulse up to 60°C (in soil) with activating dormant buds and producing new fronds.

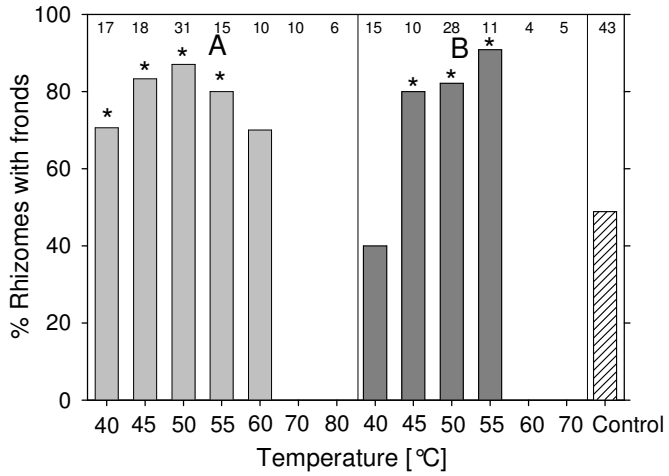

Fig. 5-6: Frond production from short shoots after heat treatment A) in soil, B) in water bath measured 8 weeks after treatment (significant differences between heat-treated rhizomes and controls were calculated by Mann-Whitney U-tests with $P < 0.05$ and are marked with an asterisk). The numbers in the top of the panels indicate the actual numbers in the respective category. Only the surviving rhizomes were counted.

Comparison Of The Effects Of Cutting And Burning On Frond Production. Fronds develop from buds on the short shoots. Production of a leaf could therefore inhibit sprouting of neighbouring buds by apical dominance (Burge & Kirkwood, 1992). To examine whether the initial burst of leaf emergence after a fire (as observed under field and laboratory conditions) could be explained only by an alleviation of apical dominance after killing of the fronds or resulted from an additional stimulation by the heat pulse, regrowth of bracken on moderately infested plots after controlled burning and cutting, as well as frond production on untreated plots, was recorded. High rates of frond emergence were observed on the burned plots (Fig. 5-7). In the first four months of regeneration, regrowth after burning was twice as much as after cutting or on the control plots. Until the end of the observation time this

difference between the controls and the burned plots leveled off. But after seven months, regrowth after cutting was half as much as after burning. Therefore, the additional stimulation of buds by the heat pulse of a fire has to be appreciated when discussing bracken control measures. In addition to an enhanced spore formation after a fire (Conway, 1957) the invasive power of bracken as a postfire colonizer (Lyon & Stickney, 1976; Gliessman, 1978) appears to rest primarily with the rhizomes, more precisely with the heat resistant and frond developing short shoots. Thus, the local practice of pasture maintenance by repeated burning, favors the fern while concomitantly weakening the pasture grasses.

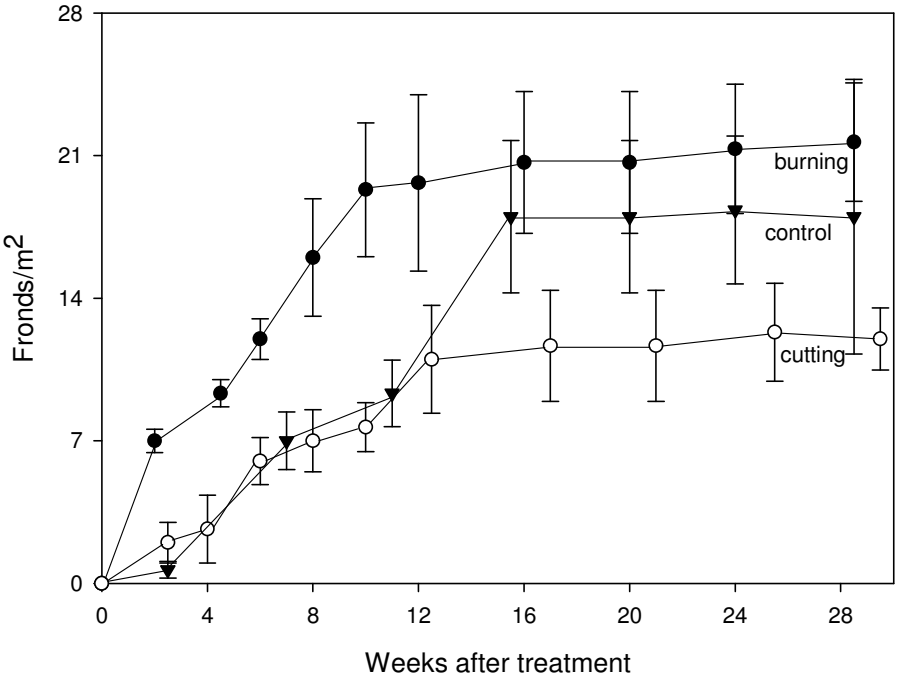

Fig. 5-7: Regrowth of southern bracken (number of newly emerged fronds, means ± SE) after cutting of all fronds, burning of the areas and without treatment (controls) on three 1 m2 plots each.

Sources of Materials

[1]automated climate station from Thies, Göttingen/Germany

[2]high resolution *P*latinum Resistance *T*hermometer with a resistance of 1000 Ω at 0°C, from HERAEUS, Hanau/Germany, self configurated

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REFERENCES

Ader KG (1990) The effect of climate and soils on bracken vigor and distribution on Scotland. In: Bracken Biology and Management (Thomas JA, Smith RT, eds) Austral Inst Agric Sci Occasional Publ. 40: 141-152.

Agee JK, Huff MH (1987) Fuel succession in a western hemlock/douglas-fir forest. Can J Forest Res 17: 697-704.

Alonso-Amelot ME, Rodulfo-Baechler S (1996) Comparative spatial distribution, size, biomass and growth rate of two varieties of bracken fern (*Pteridium aquilinum* L. Kuhn) in a neotropical montane habitat. Vegetatio 125: 137-147.

Beck E, Hartig K, Roos K (2008a) Forest clearing by slash and burn. In: Beck E, Bendix J, Kottke I, Makeschin F, Mosandl R (eds.) Gradients in a Tropical Mountain Ecosystem of Ecuador. Ecological Studies, Vol. 198. Springer, Berlin Heidelberg, pp 371-374.

Beck E, Makeschin F, Haubrich F et al. (2008b) The ecosystem (Reserva Biológica San Francisco). In: Beck E, Bendix J, Kottke I, Makeschin F, Mosandl R (eds.) Gradients in a Tropical Mountain Ecosystem of Ecuador. Ecological Studies, Vol. 198. Springer, Berlin Heidelberg, pp 1-13.

Bendix J, Silva B, Roos K, Göttlicher D, Rollenbeck R, Nauß T, Beck E (2009) Model parameterization to simulate and compare the PAR absorption potential of two competing plant species. Int. J. Biometeorol. 35, DOI 10.1007/s00484-009-0279-3.

Bray JR (1991) Growth, biomass, and productivity of a bracken (*Pteridium esculentum*) infested pasture in Marlborough Sounds, New Zealand. N Z J Bot 29: 169-176.

Brown RW (1986) Bracken in the North York Moors: Its ecological and amenity implications in National Parks. In: Smith RT, Taylor JA (eds.) Bracken: Ecology, Land Use and Control Technology. The Parthenon Publishing Group Limited, Leeds Lancs, pp 77-86.

Burge MN, Kirkwood RC (1992) The control of bracken. Crit. Rev. Biotech. 12: 299-333.

Burge MN, Irvine JA, McElwee M (1986) The potential for biological control of bracken with the causal agents of curl-tip disease. In: Proceedings of an International Conference: Bracken: Ecology, Land Use and Control Technology: 453-458.

Conway E (1957) Spore production in bracken. J Ecol 45: 273-284.

Der JP, Thomson JA, Stratford JK, Wolf PG (2009) Global chloroplast phylogeny and biogeography of bracken (*Pteridium*; Dennstaedtiaceae). Amer J Bot 96: 1041-1049.

Evans GR, Nordmeyer AH, Kelland CM (1990) Biomass and nutrient pools of bracken growing under radiate pine, Nelson, New Zealand. In: Bracken Biology and Management (Thomson JA, Smith RT eds) Australian Inst. of Agricultural Science, Sydney 40, pp 187-196.

Flinn M, Pringle J (1983) Heat tolerance of rhizomes of several understory species. Can J Bot 61: 452-457.

Flinn MA, Wein RW (1988) Regrowth of forest understory species following seasonal burning. Can J Bot 66: 150-155.

Gliessman SR (1978) The establishment of bracken following fire in tropical habitats. Amer Fern J 68: 41- 44.

Göttlicher D, Obregón A, Homeier J et al. (2009) Land-cover classification in the Andes of southern Ecuador using Landsat ETM+ data as a basis for SVAT modelling. Int J Remote Sens 30: 1867-1886.

Hamer, U, Potthast K, Makeschin, F (2009) Urea fertilisation affected soil organic matter dynamics and microbial community structure in pasture soils of Southern Ecuador. Appl. Soil Ecol. 43: 226-233.

Hartig K, Beck E (2003) The bracken fern (*Pteridium arachnoideum*) dilemma in the Andes of South Ecuador. Ecotropica 9: 3-13.

Hollinger DY (1987) Photosynthesis and stomatal conductance patterns of two fern species from different forest understoreys. J Ecol 75: 925-935.

Ingram DC (1931) Vegetative changes and grazing use on Douglas-fir cut-over land. J Agric Res 43: 387-417.

Lauer, W, Bendix J (2004) Climatology (Klimatologie). Westermann: Braunschweig. 352 p.

Le Duc MG, Pakeman RJ, Marrs RH (2003) Changes in the rhizome system of bracken subjected to long-term experimental treatment. J Appl Ecol 40: 508-522.

Lyon LJ, Stickney PF (1976) Early vegetal succession following large northern Rocky Mountain wildfires. In: Proceedings, Tall Timbers Fire Ecology Conference and Intermountain Fire Research Council Fire and Land Management Symposium, 1974, Missoula, MT. No. 14, Tallahassee, Tall Timbers Research Station: 355-373.

Marrs R, Johnson S, Le Duc M (1998) Control of bracken and restoration of heathland. VI. The response of bracken fronds to 18 years of continued bracken control or 6 years of control followed by recovery. J Appl Ecol 35: 479-490.

Page C (1976) Taxonomy and phytogeography of bracken - a review. Bot J Linn Soc 73:1-34.

Page C (1982) The history and spread of bracken in Britain. Proc Roy Soc Edinb 81: 3-10.

Page C (1986) The strategies of bracken as a permanent ecological opportunist. In: Smith RT. The Proceedings of the International Conference - Bracken ´85/ eds. Bracken: Ecology, Land Use and Control Technology: 173-181.

Pakeman R, Marrs R, Jacob P (1994) A model of bracken (*Pteridium aquilinum*) growth and the effects of control strategies and changing climate. J Appl Ecol 31: 145-154.

Pakeman R, Le Duc MG, Marrs RH (2000) Bracken distribution in Great Britain: Strategies for its control and the sustainable management of marginal Land. Ann. Bot. 85: 37-46.

Pitman JI, Pitman RM (1990) Climate and bracken growth. Pages 163-173 in: J. A. Thomson and R. T. Smith, ed. Bracken Biology and Management. Australian Inst. Agricult. Sci. Occasional Publ. 40, Sydney.

Potthast K, Hamer U, Makeschin F (2010) Impact of litter quality on mineralization processes in managed and abandoned pasture soils in Southern Ecuador. Soil Biol. Biochem. 42: 56-64.

Quitete-Portela, RC, Silva-Matos DM, Pugliese de Siqueira L *et al.* (2009) Variation in aboveground biomass and necromass of two invasive species in the Atlantic rainforest, Southeast Brazil. Acta Bot. Bras. 23: 571-577.

Roberts J, Pymar CF, Wallace JS et al. (1980) Seasonal changes in leaf area, stomatal and canopy conductances and transpiration from bracken below a forest canopy. J Appl Ecol 17: 409-422.

Rollenbeck R, Bendix J, Fabian P et al. (2007) Comparison of different techniques for the measurement of precipitation in tropical montane rain forest regions. J Atmospheric and Ocean Technol 24: 156-168.

Sharik TL, Ford RH, Davis ML (1989) Repeatability of invasion of Eastern White Pine on dry sites in Northern Lower Michigan. Am Midl Naturalist 122: 133-141.

SPSS 2004. SPSS for Windows, version 13.0. Chicago, Illinois, USA.

Stewart GH (1988) The influence of canopy cover on understory development in forests of the Western Cascade Range, Oregon, USA. Vegetatio 76: 79-88.

Thomson J (2000) Morphological and genomic diversity in the genus *Pteridium* (Dennstaedtiaceae). Ann Bot 85: 77-99.

Watt A (1940) Contributions to the ecology of bracken (*Pteridium aquilinum*).I. The rhizome. New Phytol 39: 401-422.

Watt A (1976) The ecological status of bracken. Bot J Linn Soc 73: 217-239.

Waring RH, Major J (1964) Some vegetation of the California Coastal Redwood Region in relation to gradients of moisture, nutrients, light, and temperature. Ecol Monographs 34: 167-215.

Webster B, Steeves T (1958) Morphogenesis in *Pteridium aquilinum* (L.) Kuhn. General morphology and growth habit. Phytomorphol 8: 30-41.

CHAPTER 6

Re-pasturisation of abandoned pastures in South Ecuador

Short-term and long-term effects of weed-control measures on bracken infested pastures and an attempt to regenerate abandoned pastures in South Ecuador

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Running head: Control experiment

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SUMMARY

Bracken (*Pteridium* spp.) is one of the most persistent weeds worldwide. This communication for the first time reports on experiments to control the extremely aggressive neotropical bracken, *P. arachnoideum*. In a South Ecuadorian area, where former pastures are overgrown by bracken, 13 different control treatments were examined in 6 repetitions over a time period of 23 months: Cutting of the leaves, various herbicides, covering with plastic foil, and combinations thereof. Subsequently, the pasture grass *Setaria sphacelata* was planted. Growth of bracken and later on of the grass was monitored monthly using the variables cover and height of vegetation. Bracken frond biomass was determined at the end of the control treatments. None of the treatments resulted in a complete eradication of the weed. The efficacy of the pest control treatments differed considerably but the subsequently planted grass balanced out these differences, suppressing the fern to a cover of less than 40%. Thus, in spite of the high resistance of (tropical) bracken to any kind of control, regeneration of abandoned pastures is possible, using a two-step strategy: Depleting of the reserves in the rhizomes by repeated killing of the leaves and subsequent suppression by a highly competitive pasture grass. For practical weed management three consecutive treatments with the herbicide mixture of metsulfuron methyl and picloram, or four consecutive cuts of the fronds are recommended to achieve maximum control effect.

Keywords: southern bracken, *Pteridium arachnoideum*, *Setaria sphacelata*, bracken control, pasture restoration

INTRODUCTION

Bracken (*Pteridium aquilinum* (L.) Kuhn) is a rhizomatous fern that can produce dense canopies on disturbed areas in particular where forest has been cleared (Taylor, 1985). Due to its peculiar ecological and physiological traits it is one of the most persistent weeds worldwide. These traits are: high resistance against pathogens, poor palatability for grazing animals, allelopathic effects on competing species, high potential for propagation by spores and by the long-living rhizomes, resistance to weather extremes and bush-fires, and a remarkable genetic plasticity (for review see Page, 1986). Recent reports on its advance, e.g. in Central America (Schneider, 2008) impressively demonstrate the enormous invasive power especially of the tropical bracken. *Pteridium aquilinum* (L.) Kuhn represents an aggregate, which can be differentiated into a northern and a southern group (Tryon, 1941; Page, 1976). While the northern "*aquilinum*" complex is ecophysiologically characterized by a life cycle that is synchronized by the winter break and the concomitant die-off of all leaves, the southern group (amongst others the diploid *Pteridium arachnoideum* (Kaulf.) Maxon (see Thomson *et al.*, 2008), and the allotetraploid *Pteridium caudatum* (L.) Maxon) comprises mainly tropical representatives with a year-round growth (Thomson, 2000; Thomson & Alonso-Amelot, 2002).

Control of bracken is extremely difficult requiring continuous treatment over many years (Marrs *et al.*, 1998). Moderately successful control of northern bracken has been achieved with repeated cutting of the fronds or treatment with specific herbicides or combinations of both (Pakeman *et al.*, 2000). Cutting of the fronds, however favours sprouting of new leaves by releasing resting buds on the rhizomes from apical dominance (Burge & Kirkwood, 1992). Many herbicides have been used for control of northern bracken (for overview see Burge & Kirkwood, 1992). Picloram (a systemic broadband herbicide) and metsulfuron-methyl (a systemic sulfonylurea herbicide) have proven rather effective (Farnworth & Davies, 1974; West & Richardson, 1985). Today, asulam, a systemic and selective herbicide as well as the total herbicide glyphosate are more commonly applied. With both a reduction of frond density of more than 95% in the year after the treatment was achieved (Williams &

Foley, 1975; Veerasekaran *et al.*, 1977, 1978). Whereas many studies have been conducted to control the northern bracken species, reports on control of the even more aggressive tropical bracken are still lacking.

In the Andes of South Ecuador tropical bracken readily invades areas on which the mountain rain forest has been cleared, mainly for the production of pastures. In the research area (Fig. 6-1A) where the forest has been cleared about 50 years ago (Martínez Jerves, 2007) , about 40% of the pastures are meanwhile heavily infested by bracken (Göttlicher *et al.*, 2009). Spreading of a bracken-dominated vegetation is greatly favored by the common use of fire, not only for clearing the forest but later on to stimulate rejuvenation of the grass and to control growth of bracken (Alonso-Amelot & Rodulfo-Baechler, 1996; Hartig & Beck, 2003; Beck *et al.*, 2008a). However, such bush-fires accelerate emergence of new bracken fronds (Roos *et al.*, 2010). The common non-indigenous pasture grasses *Setaria sphacelata* (Schumach.) Stapf and C.E.Hubb. ex Chipp., and *Melinis minutiflora* P. Beauv. appear as similarly fire-tolerant as bracken, however, burning and grazing together weakens their vigor and finally results in its suppression by the fern. In addition, heavily propagating shrubs, mainly of the Asteraceae and Melastomataceae, get a foothold under the bracken canopy and finally overtop not only the grass but also the fern. After all, repeated burning of the pastures results in a persistent vegetation in which bracken and shrubs balance each other (Hartig & Beck, 2003). For re-pasturisation of such useless areas, appropriate pest control measures are needed.

Here, we report on pest control experiments of southern bracken in tropical Ecuador. Short and long term effects of 13 treatments were analysed. Individual pest control treatments were for at least 23 months after which the pasture grass *Setaria sphacelata* was planted whose growth was then monitored for 18 months.

MATERIAL AND METHODS

Experimental Site. The study area is located on the northern slopes of the upper Rio San Francisco valley in the Cordillera oriental of the South Ecuadorian Andes (3°58'30'' S, 79°4'25'' W; Fig. 6-1A). It extends over a horizontal stretch of 0.3 km, and vertically from 1,900 to 2,100 m a.s.l.. The perhumid area is characterised by an almost constant annual temperature of 15.5 °C and an annual precipitation of 2050 mm (Bendix *et al.*, 2008). Parent rock material consist of various silicate minerals and the soils are predominantly cambisols (Beck *et al.*, 2008b), with a shallow, sometimes even lacking organic layer. After clearing of the forest 50 years ago, the steep slopes (between 25° and 40°) were used as pastures for about 40 years, but are now covered by a more or less closed canopy of bracken fronds, up to 2 m high, which is penetrated by scattered shrubs (Fig. 6-1B). The common practice of pasture maintenance by occasional burning (Hartig & Beck, 2003; Beck *et al.*, 2008a) has also been applied to the research area.

Experimental Design. The research area is rutted by numerous ravines, leaving in between only relatively narrow patches of a homogeneous bracken-dominated vegetation. Three contiguous areas with a bracken cover between 72 and 85% were chosen for the bracken control experiment. Average bracken frond height was 128.7 ± 1.2 cm (mean ± SE) with an initial density of 17.6 ± 0.4 fronds per m^2 (mean ± SE). Control measures were applied to sets of four quadrangular 5 × 5 m plots each, which were randomly distributed over the selected patches (Fig. 6-1D). An additional set of 4 plots without any treatment was used as a reference (reference-1). Because of a patchy occurrence of a blight disease which seriously affected these reference plots, another set of 6 untreated plots on the same slope was included as another reference (reference-2). The individual 25 m^2 plots were not separated by buffer zones since the dense network of the rhizomes did not allow identification of individual plants. Therefore the effects of the treatments could only be recorded from the reaction of the fronds.

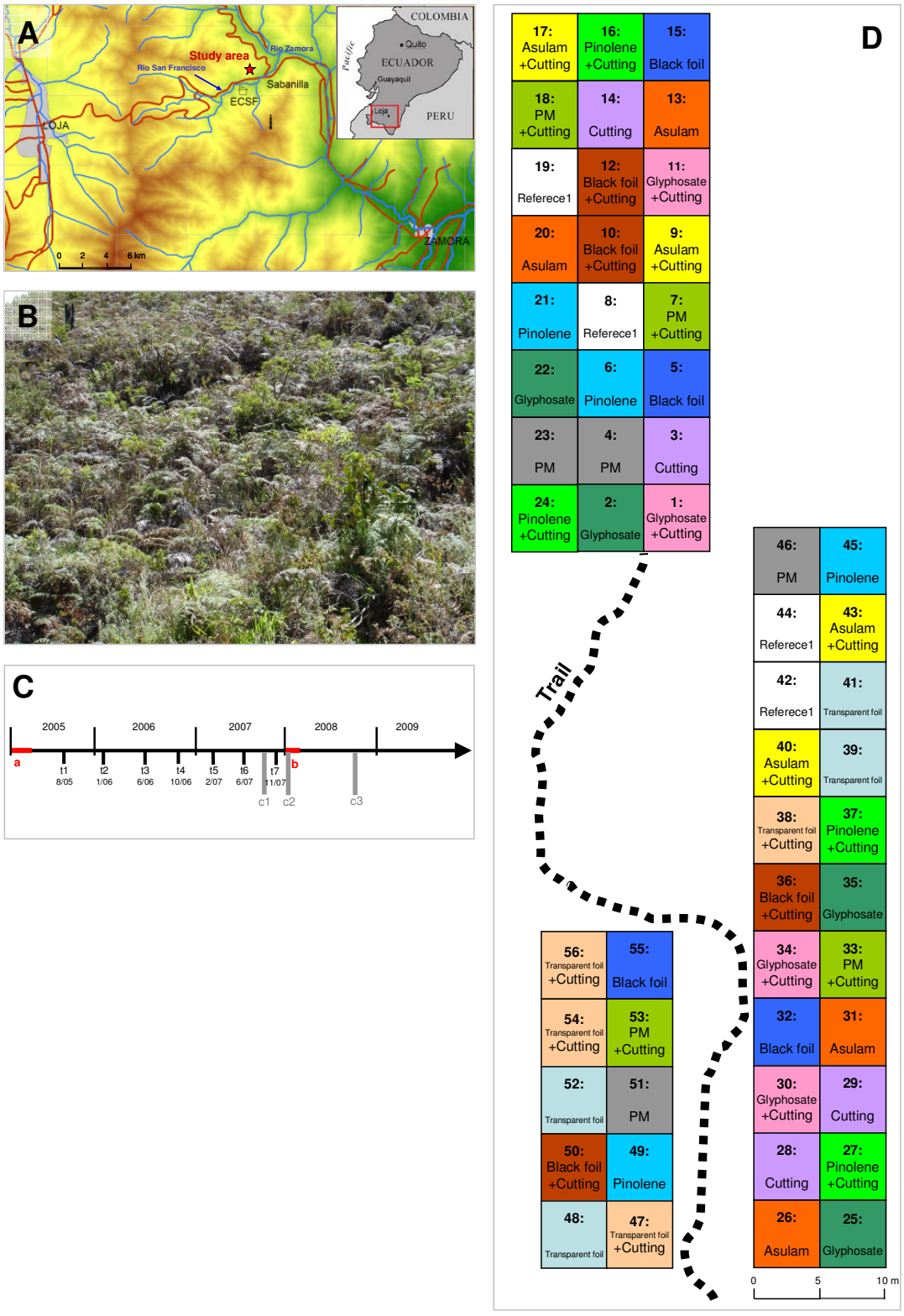

Fig. 6-1: A) Location of the research site; ECSF: the research station; B) Typical vegetation of the experimental plots prior to control treatments; C) Sequence and dates of treatments, a: installation of the experiment, t1-7: pest control treatments, b: Planting of *S. sphacelata*, c1-3: cutting of bracken fronds on the plots; D) Location of the experimental plots on the selected slope.

Bracken Control Measures. Considering results from northern bracken control experiments (Burge & Kirkwood, 1992; Marrs *et al.*, 1998; Le Duc *et al.*, 2000, 2003) the following 13 control measures were applied: (1) mechanical control by *cutting* of the fronds; (2) chemical control with herbicides: *asulam* (Asulox, 400 g a.i. L^{-1} with 6 L ha^{-1}, Bayer), *glyphosate* (Glifosato 48 SL, 480 g a.i. L^{-1} with 4 L ha^{-1}, Coagro), a herbicide combination of picloram and metsulfuron methyl (Combo, picloram: 240 g a.i. L^{-1} and metsulfuron methyl: 600 g a.i. L^{-1} with 4L ha^{-1}, Dow Agro Science, for shortness the term "*PM*" is used in the text) and *pinolene* as a transpiration blocker (Wilt Pruf, 25% solution with 6 L ha^{-1}, Wilt Pruf Products Inc.); (3) physical control by covering the plot with *black* or *transparent* plastic *foil*.

For mechanical weed control, bracken fronds were cut at the soil surface and the litter was left on the plot. Herbicide solutions were spot-sprayed on the surface of the vegetation, using a backpack sprayer. Sheets of robust *transparent* or *black* plastic *foil* of 36 m^2 size were put on top of the entire vegetation of a plot and their protruding edges were fixed with stones and tent pegs. The *foil* cover lasted for three weeks after every application date. In addition to the individual measures, combinations of herbicide treatments or foil cover with *cutting* were also applied in an alternating way. In these cases the intensities of the alternating measures were only half compared to that of the individual treatment. Depending on the weather conditions, treatments were routinely applied every 4 to 6 months for a time-period of at least 23 months (August 2005 until June 2007, Fig. 6-1C, t1 – 6). Four months after the sixth treatment (t6), the complete above-ground vegetation of all plots was cut and the fresh weight of living bracken fronds was determined (for reference-2 an area very close was chosen). Because of the restricted number of plots, biomass determination (fresh weight) could be done only once. Four weeks later, when the fern had already started to resprout the final control treatments were made (t7 in Fig. 6-1C). Treatments and monitoring of the effects were performed as cautiously as possible from the edges of the plots. Nevertheless, stepping on a leaf could not be completely avoided, but the damage was within the scope of discretion when estimating the bracken cover.

Regeneration of the Pastures. After two months of fallow, the pasture grass *Setaria sphacelata* was planted in January 2008 (b in Fig. 6-1C). Upon planting, all meanwhile regrown bracken fronds were pulled out (c2 in Fig. 6-1C). Bunches of *S. sphacelata* were obtained from local farmers, shortened to a length of 15 - 20 cm and longitudinally split in small portions of approximately 3 cm diameter. In each plot, 81 plantlets were planted in horizontal as well as vertical distances of 50 cm. Upcoming bracken fronds were cut again 9 months later (c3 in Fig. 6-1C).

Monitoring. The plots were observed in four-week intervals and bracken cover (percent of plot area) and height of the fronds was recorded. To examine the impact on other plants, mortality of 5 individuals of indigenous woody plants was also recorded in each plot. After replanting *S. sphacelata*, its cover and height were included in the observation.

Data Analysis and Statistics. The R version 2·9·0 (R Development Core Team, 2009) was applied for statistical analysis. Statistical significance of differences between the effects of treatments was routinely checked with the Kruskall-Wallis H-test and Mann-Whithney U-Test as post hoc because of non-Gaussian distribution of the data. The effects of the consecutive treatments (Fig. 6-5) were assessed by multivariate linear mixed-effects models using the lme4 package (Bates, 2005). *P*-values were calculated by likelihood-ratio tests based on differences in the deviance when each term was dropped from the full model (Faraway, 2006). Normal distribution of model residuals of all parametric models was checked visually by normal probability plots and by using the Shapiro-Wilk test. Homogeneity of variances and goodness of model fit (as suggested in Onofri *et al.*, 2010) was checked by plotting model residuals versus fitted values (Faraway, 2006). These models included a random factor coding for the date of monitoring in the different plots in order to correct for differences in the observation periods. To analyse the effects of individual subsequent control measures, the time points of the individual monthly measurements within the interval between two consecutive treatments were coded and included as a covariate. To differentiate between the results of the consecutive treatments another code was given for each application phase and

included as a fixed factor. For better visibility of differences a new response variable was calculated combining cover and vegetation height (C×H) which correlated well with biomass (n = 56, r_S = 0.946, P < 0.001). Because of a non-Gaussian distribution it was square-root transformed to adjust it to a normal distribution. The entire data set of each treatment was separated into the six application phases (after applications t1-t6, Fig. 6-1C) and every two consecutive phases were compared with each other.

To examine the effect of bracken control measures on the woody plants in the plots the percentages of the dead individuals were compared at the end of each post-treatment interval and differences between the most effective control treatments (and reference-1) were analysed.

RESULTS

Effectiveness of Control Treatments. After six consecutive treatments bracken cover was decreased to less than 20% of the initial values irrespective of the applied method (Fig. 6-2A). *PM*, *cutting*, *glyphosate*, *asulam* and *PM + cutting* were the most effective treatments, resulting in bracken covers less than 10%. Vitality of the fern, as indicated by the size (height) of the new fronds was strongly affected by the treatments: *asulam + cutting*, *cutting*, *PM + cutting*, *black foil + cutting* , *black foil* and *transparent foil + cutting* which all resulted in a decrease by about 40% (Fig. 6-2B).

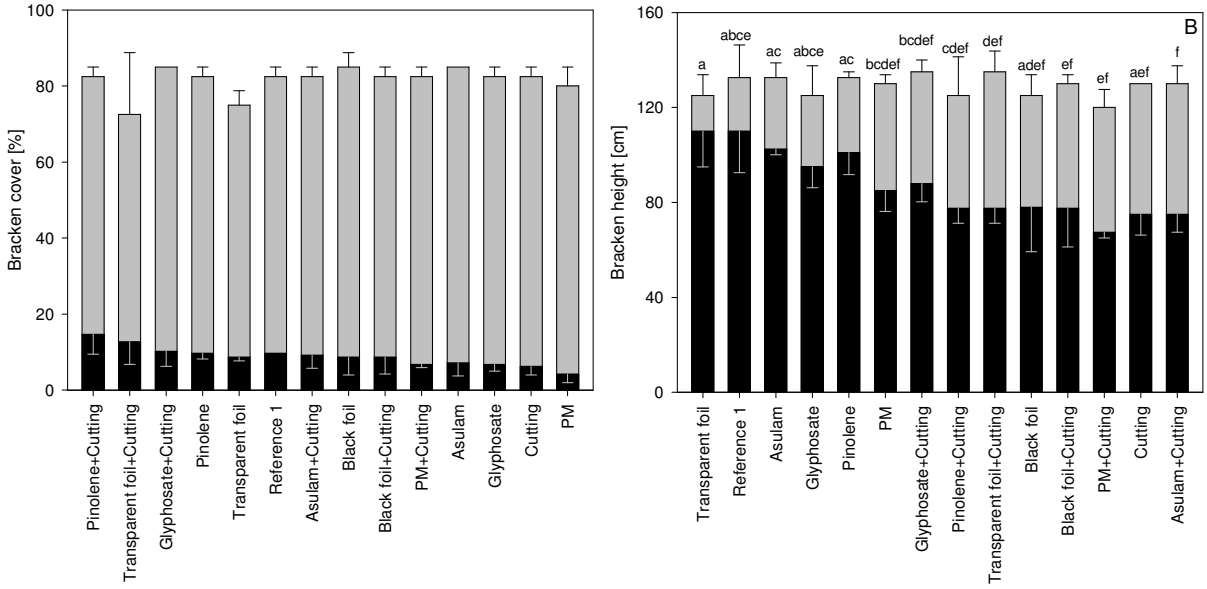

Fig. 6-2: Effects of 6 consecutive bracken control treatments on bracken cover (A; percental changes χ^2 = 19.29, *df* = 13, *P* = 0.11) and height of the bracken fronds (B; percental changes χ^2 = 32.71, *df* = 13, *P* < 0.05); Initial cover and height of bracken in grey and values after the 6 treatments in black; medians with 75th and 25th percentiles of the 4 plots per treatment are shown. Different letters indicate significant differences between the effects of treatments (Mann-Whitney-U post-hoc for significant Kruskall-Wallis tests, α = 0.05).

Above-ground biomass of bracken fronds from untreated reference-2 area ranged between 820 and 1000 g fresh weight per m^2. After 2 years of consecutive treatments biomass of the fronds was reduced to values between 800 and 240 g per m^2 (Fig. 6-3). Most effective were the treatments: *cutting* and *PM*, resulting in the lowest above-ground biomass of 240 and 260 g per m^2, respectively. Only little less effective proved the treatments *black foil + cutting*, *PM + cutting*, *black foil* and *asulam* (fresh weights of 415 to 480 g per m^2).

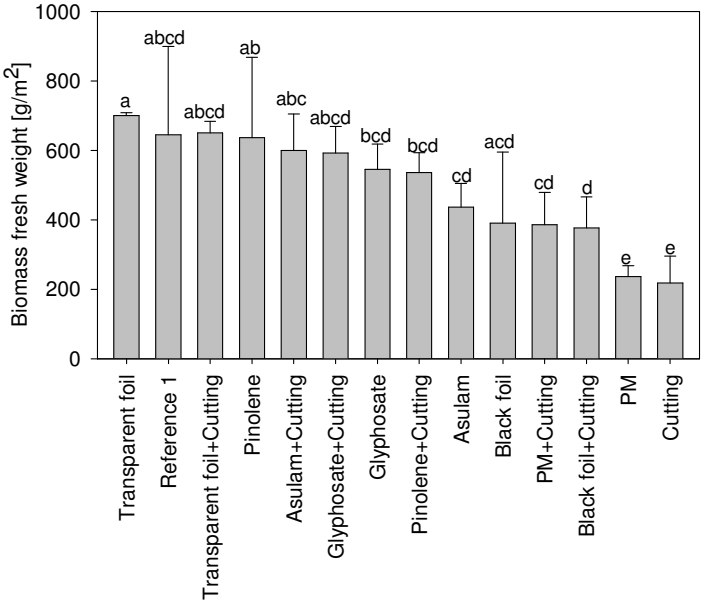

Fig. 6-3: Effects of 6 consecutive bracken control treatments on the above-ground bracken biomass (χ^2 = 38.54, *df* = 13, *P* < 0.001). Medians with 75th and 25th percentiles of the 4 plots per treatment are shown. Different letters indicate significant differences (Mann-Whitney-U post-hoc α = 0.05).

Reference Plots. On the untreated reference-1 plots bracken cover fluctuated between 71 and 98% during the initial ten months of observation (December 2004 to September 2005). Such slight fluctuations were observed also in the reference-2 plots throughout the whole experiment (Fig. 6-4) where, however, the number of fronds was determined instead of frond cover. Small fluctuations are typical of undisturbed growth. Frequently, dying of old leaves coincided temporally with the emergence of new ones and the average rates of both processes were nearly 1 : 1. Different from such small changes, a patchy infection by pathogenic fungi from time to time affected part of the leaves, triggering a sudden breakdown of the bracken canopy. The dominant pathogenic fungus has been tentatively identified as *Ascochyta pteridis* Bres. (R. Berndt, Swiss Federal Institute of Technology, Zurich/Switzerland, pers. comm.). In October 2005, such an infection reduced the bracken cover on the reference-1 plots down to 12%. However, the effect of this infection was limited to the untreated plots, since the fronds of the treated plots were either not present (after *cutting*) or were killed by the herbicides or under the *foil* cover. Partial recovery from the fungal infection to a coverage of about 50% required

two months. Thirteen months after the first infection (November 2006), a second wave of frond breakdown reduced the cover on the reference-1 plots to a minimum of 10%. Two months later, in January 2007 the bracken canopy had recovered to a cover of 32%. Reference-2 plots were not affected by the blight.

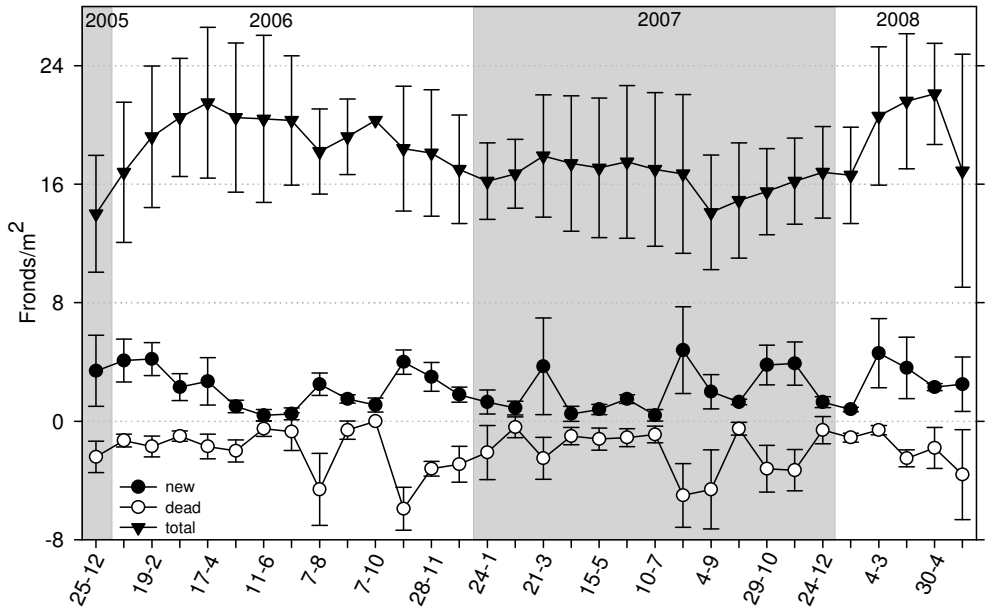

Fig. 6-4: Bracken frond dynamics on plots of reference-2 over a time period of 2.5 years, determined monthly by total number, and the numbers of emerging and dying fronds (mean ± SE per m^2; n = 6 plots).

Effects of Consecutive Treatments. For further analysis only the six most effective control measures *PM*, *cutting, black foil*, *asulam* and the combinations *PM +cutting* and *black foil + cutting* were used.

For practice, it is important to know the minimum of consecutive treatments required for weakening bracken sufficiently for successful re-pasturisation. While all control measures finally resulted in a reduction of the bracken cover by more than 80% (Fig. 6-2A) pronounced differences between the various treatments were observed on a short-term level after the individual treatments. Figure 6-5 shows regrowth of bracken in the intervals between two consecutive treatments. Maximum effect of a treatment was attained when the effects of 2 subsequent treatments were

identical. Time series of bracken regrowth after each treatment were calculated and the differences between 2 consecutive phases were statistically analysed. In addition to the number of consecutive treatments required for the maximum effect, the extent of the effects is important. For better visualisation of the effects a new response variable [cover × vegetation height] was calculated. After the first application of *PM* only a slight reduction in bracken cover and frond height was visible, but the second application resulted in a total breakdown from which the weed did not recover (Fig. 6-5). After 3 consecutive treatments the maximum effect was achieved. Likewise effective was *PM + cutting*. Slight regeneration was observed after each of the first 4 *cuttings*, but subsequently bracken regrowth was very low. Immediate killing of the leaves was also achieved with *asulam*, however the long-term effect was smaller than with *PM* or *cutting*. *Black foil* and the combination *black foil + cuttin*g resulted in a gradually increasing effect by the initial 4 treatments, but again the overall impact was less than of the other treatments.

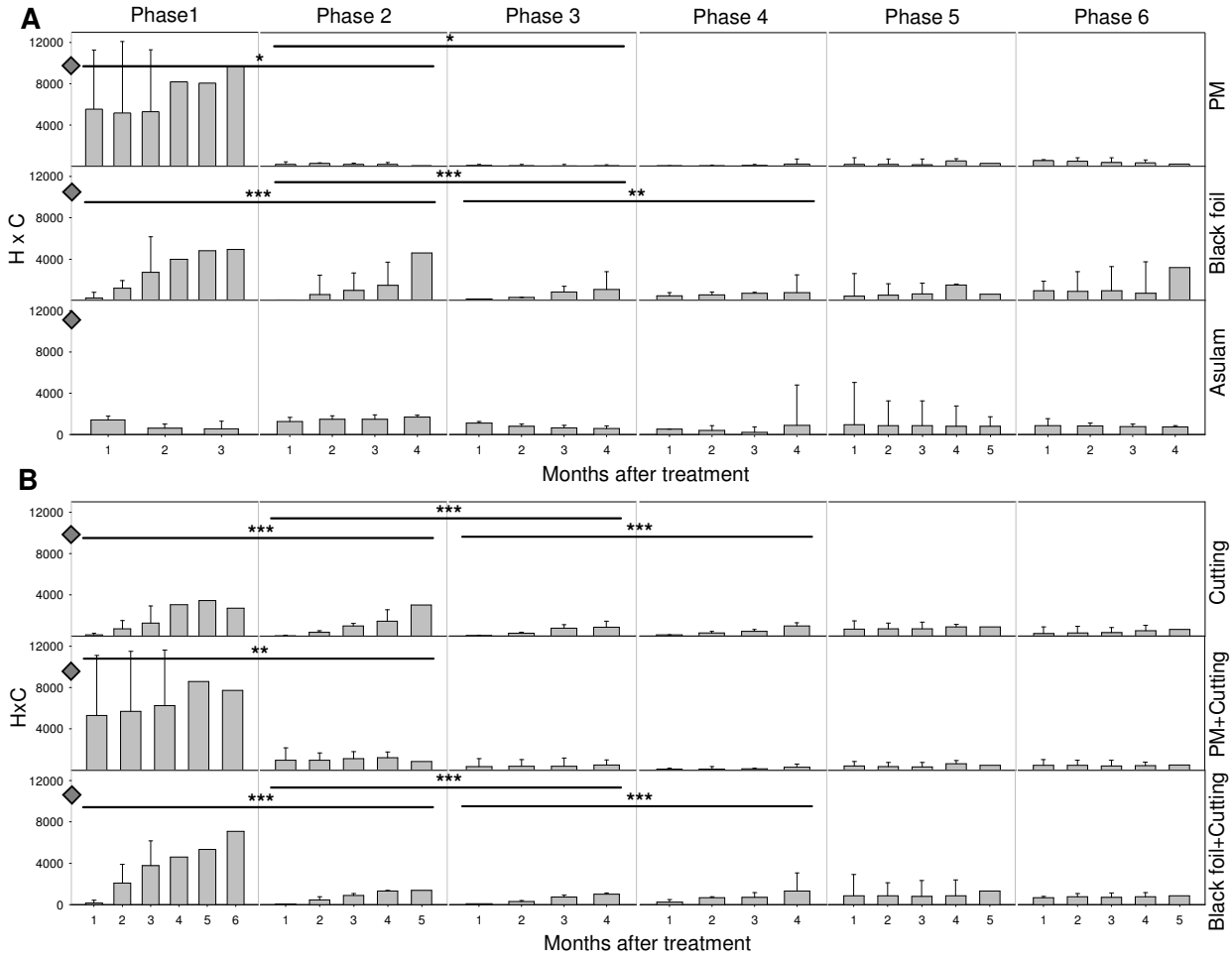

Fig. 6-5: Regrowth of bracken (cover × height, for explanation see material & methods) during the intervals of the 6 consecutive applications of the most effective control treatments. The combination treatments *black foil + cutting,* and *PM + cutting* started in the first phase with foil and the herbicide, respectively, followed by cutting in the next phase and so on. Medians with 75th and 25th percentiles are shown. Initial values are indicated by a grey diamond on the Y-axis. Horizontal bars mark significant differences between two consecutive phases as analysed by linear mixed effects models (significance levels: $P < 0.01$ *, $P < 0.001$ **, $P < 0.0001$ ***).

Impact on Woody Species. *Cutting* was not applied to the woody plants on the plots. Again spraying *PM* was the most effective treatment which resulted in less than 10% survivors after 2 years (Fig. 6-6). *PM + cutting* (of the fern) and covering with *black foil* killed between 70 and 75% of the shrubs and young trees. *Cutting* (of the fern), *black foil + cutting,* and *asulam* were not effective ($P > 0.05$). In the combination *PM + cutting* the amount of the applied herbicide was only 50%, and

lowering of the competitive pressure by cutting the bracken fronds could have fostered growth of the shrubs. Dieback of shrubs and young trees in the reference-1 plots represented the natural mortality because the blight selectively affects bracken. Analysis of short-term effects showed that *PM* immediately strongly affected the woody plants (over 70% mortality after the first treatment, Fig. 6-6). Less than 10% of the shrubs and young trees survived the fifth treatment. *PM + cutting* as well as covering with *black foil* were initially much less effective but a gradual increase was observed with the following 4 treatments.

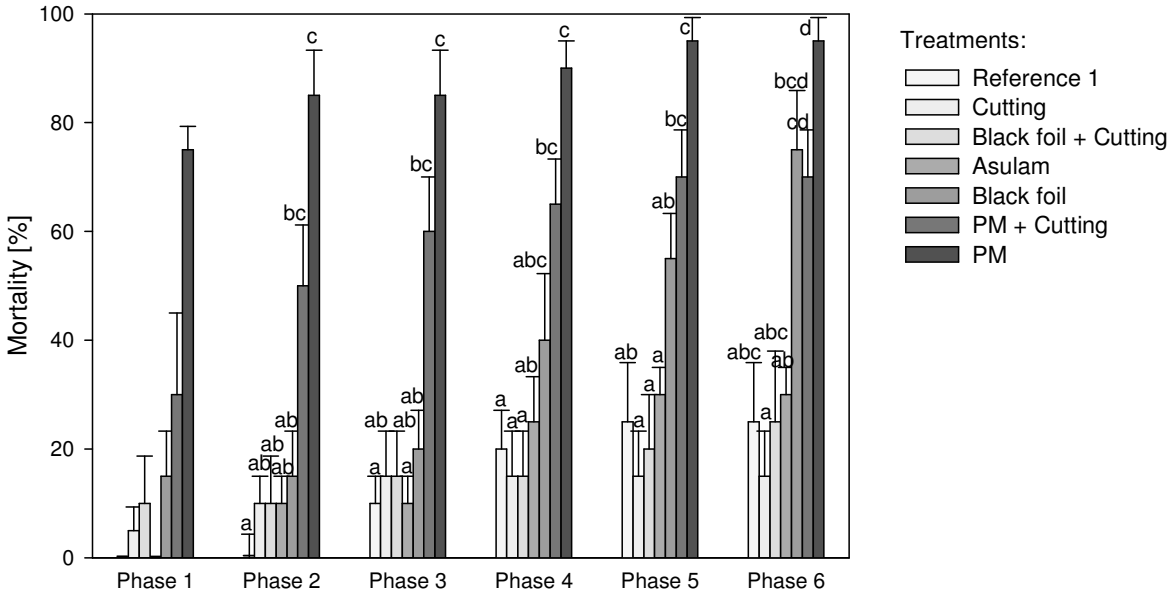

Fig. 6-6: Impact of the 6 most effective bracken control treatments on shrubs and tree saplings on the experimental plots (Kruskall-Wallis (all *df* values = 6) for phase 1: $\chi^2 = 11.66$, , $P = 0.07$; phase 2: $\chi^2 = 17.54$, $P < 0.05$; phase 3: $\chi^2 = 17.29$, $P < 0.05$; phase 4: $\chi^2 = 16.99$, $P < 0.05$; phase 5: $\chi^2 = 18.86$, $P < 0.05$; phase 6: $\chi^2 = 18.85$, $P < 0.05$). Medians with 75th and 25th percentiles of 20 shrubs per treatment (5 per individual plot) are shown. Different letters indicate significant differences between the treatments (Mann-Whitney-U post-hoc for significant Kruskall-Wallis tests, $\alpha = 0.05$).

Growth of *Setaria sphacelata* and Regrowth of Bracken. After the final treatment the plots were left fallow for two months before *Setaria sphacelata* was planted. Less than 3% of the grass plantlets did not survive the transplantation but it took about three months until it was vigorously growing. There was no correlation between the

initial dying of the plantlets and the previous treatments of the plots. Concomitantly with the growth of *S. sphacelata*, resprouting of bracken was observed but at reduced rates. After the initial 6 months, slight effects of the previous treatments on the growth of bracken and *S. sphacelata* were seen, but these were statistically not significant (bracken [C×H]: $\chi^2 = 3.71$, $df = 6$, $P > 0.7$; grass [C×H]: $\chi^2 = 6.08$, $df = 6$, $P > 0.4$). Eighteen months after planting *S. sphacelata*, these differences had disappeared and all plots were clearly dominated by the grass which on average covered 73% of the plot areas while the average cover by the fern was 36%. With an average leaf height of 69.4 ± 1.3 cm (mean ± SE), *S. sphacelata* overtopped the bracken fronds [64.7 ± 1.3 cm (mean ± SE)] (Fig. 6-7).

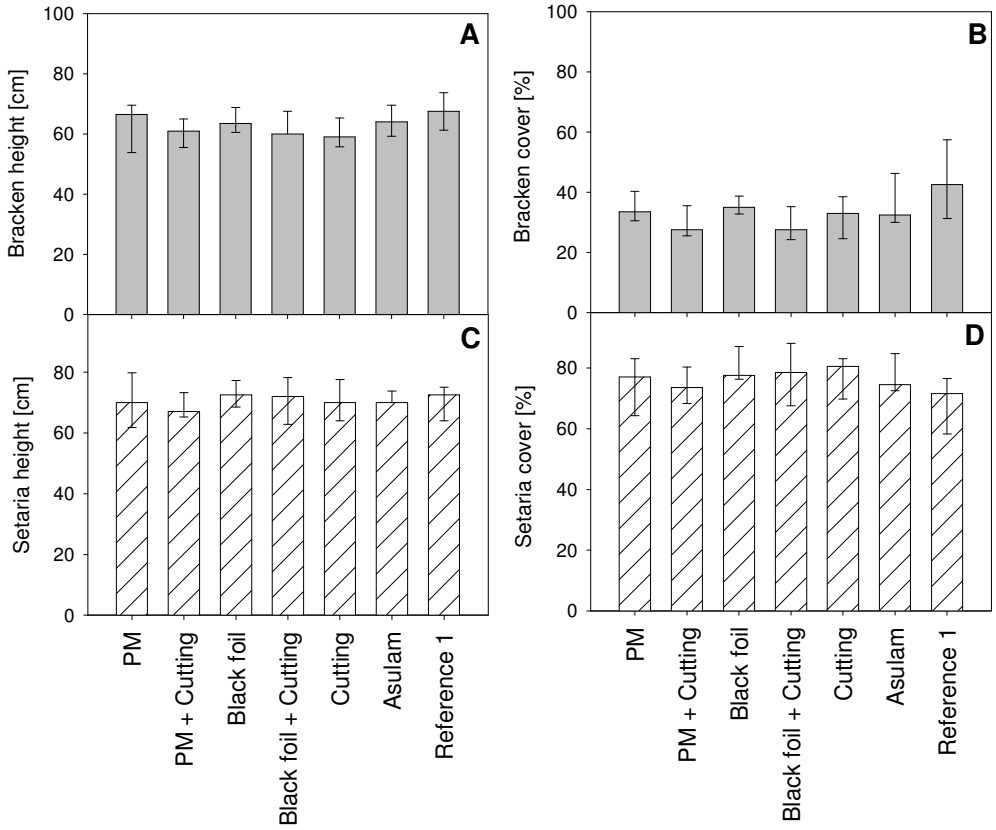

Fig. 6-7: Percent cover (A), and height (B) of bracken, and of *S. sphacelata* (C, D) after 1.5 years of re-pasturisation. Medians with 75th and 25th percentiles are shown. Differences were neither significant for bracken cover ($\chi^2 = 6.85$, $P > 0.3$) and height ($\chi^2 = 5.11$, $P > 0.5$), nor for *S. sphacelata* cover ($\chi^2 = 4.83$, $P > 0.3$) and height ($\chi^2 = 1.67$, $P > 0.9$); (all *df* values = 6).

DISCUSSION

Reference Plots. During the first ten months, slight fluctuations in bracken cover on the reference-1plots confirmed the long-term 1:1 ratio of frond emergence and frond dying of the tropical bracken. The two collapses in October 2005 and November 2006 on these plots resulted from outbursts of pathogenic fungi whose mycelia cover the fronds and cause their premature death. In the research area, fungal infection is permanently present at a low level from which incidental outbreaks of the pathogens can be expected. The ascomycete *Ascochyta pteridis* is known as parasite of the northern bracken (Burge & Kirkwood, 1992; Womack & Burge, 1993) and its detection in the tropics is of special interest with respect to possible biological control. Natural breakdown has also been reported in studies with northern bracken (cf. Marrs et al., 1998; Le Duc et al., 2003), and the authors hypothesized that periods of increased rainfall or cold may have triggered such sporadic events. Blight did not affect our reference-2 plots and it is important to note that pathogen outbreaks did not interfere with the treatments on the plots which killed the potential host leaves or sealed their surface with pinolene.

Effectiveness of Bracken Treatments. As a result of many studies on northern bracken (Marrs et al., 1998) complete eradication by above-ground control treatments is almost impossible. Therefore, a realistic aim is to maintain the degree of infestation at a low level at which it does not seriously affect growth of the useful plants. Compared to the studies with northern bracken the present investigation had to overcome three additional problems: The all year growth period of the tropical bracken, the perhumid climate and the steep slopes of the research area. To avoid washing out of the pesticides by the frequent rains moderate concentrations of the herbicides as suggested by the manufacturers were applied. The frequency of the treatments was adjusted to the economic situation of the farmers. Admittedly, covering bracken-dominated vegetation with plastic foil is not a commonly used method. Plastic foil, irrespective of transparent or black, withholds precipitation and thus imposes a strong drought effect. Under the transparent foil the light energy is converted to heat at the surface of the vegetation which results in additional heat

stress. Under black foil drought stress was aggravated by deficiency of light. Both stress combinations greatly reduced the vitality of the plants. Nevertheless, heat stress, although affecting the existent leaves, can even trigger sprouting of dormant buds on the rhizomes (Roos *et al.*, 2010). Black foil was included for comparison with cutting of the fronds which may activate dormant buds on the rhizomes (Burge & Kirkwood (1992). Upon removal of the black foil after three weeks emerging leaves due to etiolement died from the sudden exposure to light, while older leaves had already dried up under the rain shield. In summary, covering with black foil had a much stronger impact than roofing with transparent plastic.

Cutting of the fronds is a well known method for bracken control which has been used in most bracken eradication experiments as discrete measure or in combination with herbicides (Lowday, 1987; Pakeman *et al.*, 2002; Godefroid *et al.*, 2007; Stewart *et al.*, 2008). Even after three consecutive cuts, regrowth of bracken was substantial and only after the fourth cut the maximum effect was achieved (Fig. 6-5). In the long run, regular *cutting* was as effective as the best herbicide *PM* (Fig. 6-3). Combinations of other treatments with *cutting* were only initially more effective than *cutting* alone. This might be attributable to the fact that the intensity of both *cutting* and herbicide treatment in the combination was only half compared to the discrete treatments.

For recovery of abandoned areas *PM*, a customary local mixture of two systemic herbicides was most effective. This mixture warrants a broad-band effect which is important with respect to a potential genetic heterogeneity (Sheffield *et al.,* 1989) of bracken and to the accompanying woody vegetation (Figs. 6-3 and 6-6). Although *PM* is commonly used in the country, publications on its effects in bracken control are still lacking.

Glyphosate was effective in reducing bracken cover, but with a low inhibitory effect on frond production and thus on above-ground biomass production (Figs. 6-2 and 6-3). The low efficacy could be due to a low concentration which, however, was recommended by the producer. With the applied concentration (480 g a.i. L^{-1} with

4 L ha^{-1}) Burge & Kirkwood (1992) achieved up to 97% frond reduction after one application.

Asulam is a very popular herbicide in northern bracken control (e.g. Le Duc *et al.*, 2000; Petrov & Marrs, 2001). The present report is the first about *asulam* application on southern bracken showing its efficacy also under tropical conditions. The applied amount (400 g a.i. L^{-1} with 6 L ha^{-1}) was in the range of the producer's recommendations, and the lowest concentration suggested by Burge & Kirkwood (1992) for a 90 – 95% reduction of bracken frond cover. A higher concentration may be appropriate for the tropical bracken, however, it should be mentioned that these authors recommended such concentration for a one-time application whereas in this study the herbicide was applied twice or thrice per year.

Pinolene is known as a biologically innoxious terpenoid which forms a coat on the leaf surface and thus seals the stomates. It proved less effective as herbicide for bracken possibly because of the difficulty to achieve a continuous layer on the pinnate leaf surfaces.

Effects of Consecutive Treatments. Given that a complete eradication of bracken is not achievable, the minimum number of treatments required for a sustainable suppression of the fern is of economic interest. For the 6 most effective treatments the minimum number of repetitions were between one (*asulam*) and four (*PM+cutting*, *PM*, *cutting, black foil*, *black foil + cutting*). Achievement of a steady level of bracken infestation is basically independent of the degree to which bracken can be finally eliminated by a particular treatment. Thus, suppression with *PM* alone is more effective but requires one treatment more than the combination of *PM + cutting* (Fig. 6-5). The immediate effect of *asulam* could not be maintained during the following applications and therefore a minimum number of consecutive treatments could not be compiled. It must be stressed in that context that the results relate to the clearing of abandoned tropical pastures from bracken and not to the maintenance of bracken-infested pastures.

Effectiveness on Woody Species. Assuming competition between the fern and the scattered shrubs and tree saplings, measures that selectively impaired the fern could have been beneficial for the woody plants. There is a basic rate of natural die-back of about 20% which could be attributed to that competition (Fig. 6-6). *Cutting* of the bracken fronds reduced that rate and in particular decreased the effect of the otherwise most effective *PM*-treatments.

Regeneration and Maintenance of Pastures. After gaining a foothold on the cleared plots *Setaria sphacelata* grew vigorously in spite of resprouting bracken fronds. Initial differences in growth could be interpreted as memory effects of the preceding treatments on either the grass or the fern. Since these differences did not comply with the former treatments, growth inhibition by remnants of herbicides in the soil is very unlikely. Equalization of those differences, and its final dominance evidence the extraordinary competitive strength of this grass. Surprisingly the method of bracken control did not play a significant role in the final success of re-pasturisation. Once growth of the fern was markedly impaired, *S. sphacelata* took over, and successfully suppressed the fern to a level at which the yield of the pasture is not substantially compromised. The only differences of the various examined control measures arose from the efficacy of the methods and the number of treatments which are necessary for a substantial weakening of the fern.

Although the fern could not overtop the grass during the one and a half years period of observation, some weeding of the pastures is advisable. For this purpose and for rejuvenation of the tall grass bunches farmers commonly burn the pastures from time to time. As described elsewhere this measure benefits the bracken more than the grass (Roos *et al.*, 2010), and in the long run results in abandonment of the pastures (Hartig & Beck, 2003). Since application of herbicides on an active pasture is problematic, cutting of both the fern and the grass would be a measure which warrants sustainable use of the *S. sphacelata* pastures in South Ecuador.

In a re-pasturisation experiment in Great Britain, Lowday and Marrs (1992) observed an only two-year suppression of bracken after the establishment of

grasses. Similar results were reported by Le Duc *et al.* (2000), however with an even shorter suppression of bracken. These authors recommended two cuttings of (Northern) bracken per year together with careful grazing. Considering the enormous competitive strength of *Setaria sphacelata* in tropical South Ecuador in connection with the all-year growing season, one cut per year and a moderate grazing intensity could be a useful protocol for keeping the recovered pastures in good condition.

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REFERENCES

Alonso-Amelot ME, Rodulfo-Baechler S (1996) Comparative spatial distribution, size, biomass and growth rate of two varieties of bracken fern (*Pteridium aquilinum* L. Kuhn) in a neotropical montane habitat. Vegetatio 125: 137-147.

Bates D (2005) Fitting linear mixed models in R. *R* News 5, 27-30. See http://cran.r-project.org/doc/Rnews/Rnews_2005-1.pdf for more information.

Beck E, Hartig K, Roos K (2008a) Forest clearing by slash and burn. In: Beck E, Bendix J, Kottke I, Makeschin F, Mosandl R (eds.) Gradients in a Tropical Mountain Ecosystem of Ecuador. Ecological Studies, Vol. 198. Springer, Berlin Heidelberg, pp 371-374.

Beck E, Makeschin F, Haubrich F et al. (2008b) The ecosystem (Reserva Biológica San Francisco). In: Beck E, Bendix J, Kottke I, Makeschin F, Mosandl R (eds.) Gradients in a Tropical Mountain Ecosystem of Ecuador. Ecological Studies, Vol. 198. Springer, Berlin Heidelberg, pp 1-13.

Burge MN, Kirkwood RC (1992) The control of bracken. Crit Rev Biotech 12: 299-333.

Farnworth J, Davies GM (1974) The response of hill bracken and the associated pasture to applications of picloram and dicamba. Weed Res 14, 401-404.

Faraway J (2006) Extending the linear model with *R.* Chapman and Hall/CRC, New York, USA.

Godefroid S, Monbaliu D, Massant W, Van der Aa B, De Vos B, Quivy V, Koedam N (2007) Effects of soil mechanical treatments combined with bramble and bracken control on the restoration of degraded understory in an ancient beech forest. Ann of Forest Sci *64*, 321-331.

Göttlicher D, Obregón A, Homeier J et al. (2009) Land-cover classification in the Andes of southern Ecuador using Landsat ETM+ data as a basis for SVAT modelling. Int J Remote Sens 30: 1867-1886.

Hartig K, Beck E (2003) The bracken fern (*Pteridium arachnoideum*) dilemma in the Andes of South Ecuador. Ecotropica 9: 3-13.

Le Duc MG, Pakeman RJ, Putwain PD, Marrs RH (2000) The variable responses of bracken fronds to control treatments in Great Britain. Ann Bot 85, 17-29.

Le Duc MG, Pakeman RJ, Marrs RH (2003) Changes in the rhizome system of bracken subjected to long-term experimental treatment. J Appl Ecol 40: 508-522.

Lowday JE (1987) The effects of cutting and asulam on numbers of frond buds and biomass of fronds and rhizomes of bracken *Pteridium aquilinum*. Ann Appl Biol 110, 175-184.

Lowday JE, Marrs RH (1992) Control of bracken and the restoration of heathland.1. Control of bracken. J Appl Ecol 29, 195-203.

Marrs R, Johnson S, Le Duc M (1998) Control of bracken and restoration of heathland. VI. The response of bracken fronds to 18 years of continued bracken control or 6 years of control followed by recovery. J Appl Ecol 35: 479-490.

Martínez Jerves JA (2007) A vegetation analysis of secondary mountain rain forests in southern Ecuador: Effects of the mode of impact on the floristic composition and forest structure. PhD thesis, University of Bayreuth, Germany.

Onofri A, Carbonell EA, Pieho H-P, Mortimer AM, Cousens RD (2010) Current statistical issues in Weed Research. Weed Res 50, 5-24.

Page C (1976) Taxonomy and phytogeography of bracken - a review. Bot J Linn Soc 73: 1-34.

Page C (1986) The strategies of bracken as a permanent ecological opportunist. In: Smith RT. The Proceedings of the International Conference - Bracken ´85/ eds. Bracken: Ecology, Land Use and Control Technology: 173-181.

Pakeman R, Le Duc MG, Marrs RH (2000) Bracken distribution in Great Britain: Strategies for its control and the sustainable management of marginal Land. Ann Bot 85: 37-46.

Pakeman RJ, Thwaites RH, Le Duc MG, Marrs RH (2002) The effects of cutting and herbicide treatment on *Pteridium aquilinum* encroachment. Appl Veg Sci 5, 203-212.

Petrov P, Marrs RH (2001) The reclamation of bracken-dominated pastures in Bulgaria using asulam and fertilizers. Grass Forage Sci 57, 131-137.

R Development Core Team (2009) *A Language and Environment for Statistical Computing.* R Foundation for Statistical Computing, Vienna, Austria. See http://www.R-project.org. for more information.

Roos K, Rollenbeck R, Peters T, Bendix J, Beck E (2010) Growth of Tropical Bracken (*Pteridium arachnoideum)*: Response to Weather Variations and Burning. accepted for publication in Invasive Plant Sci and Management, DOI: 10.1614/IPSM-D-09-00031.1.

Schneider LC (2008) Plant invasions in an agricultural frontier: Linking satellite, ecological and household survey data. In: *Land Change Science in the Tropics - Changing Agricultural Landscapes* (eds A Millington & W Jepson), 117-141. Springer, US.

Sheffield E, Wolf PG, Haufler CH (1989) How big is a bracken plant. Weed Res 29, 455-460.

Stewart G, Cox E, Le Duc M, Pakeman R, Pullin A, Marrs R (2008) Control of *Pteridium aquilinum*: Meta-analysis of a multi-site study in the UK. Ann Bot 101, 957-970.

Taylor JA (1985) Bracken fern *Pteridium aquilinum* encroachment rates in Britain. Soil Use and Management 1, 53-56.

Thomson J (2000) Morphological and genomic diversity in the genus *Pteridium* (Dennstaedtiaceae). Ann Bot 85: 77-99.

Thomson JA, Alonso-Amelot ME (2002) Clarification of the taxonomic status and relationships of *Pteridium caudatum* (Dennstaedtiaceae) in Central and South America. Bot J Linn Soc 140, 237-248.

Thomson JA, Mickel JT, Mehltreter K (2008) Taxonomic status and relationships of bracken ferns (*Pteridium*: Dennstaedtiaceae) of Laurasian affinity in Central and North America. Bot J Linn Soc 157, 1-17.

Tryon RM (1941) A Revision ofc the Genus *Pteridium*. *Rhodora* ***43***, 1-31 and 37-67.

Veerasekaran P, Kirkwood RC, Fletcher WW (1977) Studies on mode of action of asulam in bracken (*Pteridium aquilinum* L. Kuhn). 1. Absorption and translocation of [asulam-c-14]. Weed Res 17, 33-39.

Veerasekaran P, Kirkwood RC, Fletcher WW (1978) Studies on mode of action of asulam in bracken (*Pteridium aquilinum* L. Kuhn).3. Longterm control of field bracken. Weed Res 18, 315-319.

West TM, Richardson WG (1985) Response of bracken (*Pteridium aquilinum*) in pots to various herbicide treatments. Tests of Agrochem and Cultivars 6, 108-109.

Williams GH, Foley A (1975) Effect of herbicides on bracken rhizome survival. Ann Appl Biol 79, 109-111.

Womack JG, Burge MN (1993) Mycoherbicide formulation and the potential for bracken control. Pesticide Sci 37, 337-341.

APPENDIX

Permanent removal of the forest: Construction of roads and power supply lines

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Chapter 27
Permanent Removal of the Forest: Construction of Roads and Power Supply Lines

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27.1 Introduction

The construction of roads and power supply lines causes disturbances which differ from forest clearing for farming purposes by several major issues:

1. The disturbance is linear and narrow but extends over a wide distance.
2. Not only is the topsoil affected but usually the entire soil layer is stripped and, especially in a mountainous area, even the slopes are clipped and ablated and broadenings are banked.
3. The forest is usually cleared mechanically and not by the use of fire.
4. Once the traffic route is completed, disturbance continues either by regular cutting of the vegetation along the wayside or by landslides of various dimensions caused by erosion of the steep embankments.

In the area of the RBSF several campaigns of road construction are recorded as well as the establishment of a power supply line from the neighboring hydroelectrical power plant to the capitals of Loja and Zamora. Remnants of an ancient trail traversing the forests of the RBSF at about 2100–2300 m can be detected – presumably the former connection route between both provincial capitals. The area is meanwhile completely covered by forest, but there are still indications (charcoal, pollen from cereals) that homesteads or rest houses had been put up along the trail.

27.2 The Study Area

In 1962 the former country road from Loja (2100 m) via the pass El Tiero (2700 m) was closed and a new route was established for the new road in the upper Rio San Francisco valley. Several landslides had buried the old road but unfortunately landslide activity also severely affects the new country road, especially during the heavy rains. The road surface of the old road is mostly sealed gravel. Therefore break-up of the road surface and regeneration of a

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vegetation cover can be monitored. Lichens, liverworts and mosses are the first colonizers, and from the vegetation of the roadside also vascular plants, mostly rhizomatous grasses like *Cortaderia* cf. *jubata* or *Melinis minutiflora* invade the planum. Distinctive components of the initial stages are the lichens *Dictyonema glabratum*, *Cladonia* cf. *cervicornis* ssp. *verticillata* and *Stereocaulon* sp., the liverworts *Riccardia amazonica* and *Marchantia chenopoda* and the moss *Pogonatum tortile*. Creeping pteridophytes, e.g. *Lycopodium magellanicum* and *Lycopodiella glaucescens* and the Gleicheniaceae *Sticherus* spp are characteristic of later successional stages. Colonization of the sealed planum takes a relative long time, due to the compressed substrate and recurrent stepping by the local people.

27.3 Results and Discussion

27.3.1 The Vegetation of the Embankments

The vegetation cover of the road and trail embankments is very variable and an interpretation of apparent types as successional stages in the sense of a space-for-time substitution is debatable. Great differences exist between the upper and the lower embankments as the upper resembles rather the slide face of a landslide, while the lower can be compared with a mudflow. On the upper face, the soil material is often completely removed and rocky material or compacted gravel forms the surface. Due to heavy erosion, formation of a plant cover is extremely slow and, even after 40–50 years only patchy vegetation, dominated by cryptogams, is observed. At the same time, a dense bush cover or even a secondary forest may have developed on the soil material heaped up on the lower embankment.

27.3.2 Colonization of Bare Ground

Bryophytes and lichens (e.g. *Dictyonema glabratum*, Fig. 27.1) are the first cryptogams which get a foothold on bare ground and dominate the initial phase of colonization. Liverworts (e.g. *Riccardia amazonica*) are the typical colonizers of moist and shaded places while mosses and lichens prefer more dry and open conditions.

27.3.2.1 Liverworts and the Role of Mycorrhization

Mycorrhizal fungi with their fine and dense hyphal network strongly influence the soil structure, e.g. glomeromycote fungi produce the glycoprotein glomalin that

Fig. 27.1 *Dictyonema glabratum*, growing in a carpet of the moss *Pogonatum tortile* on a road embankment in the San Francisco valley at 1850 m a.s.l

agglutinates soil particles (Frei et al. 2003; Piotrowski et al. 2004). Among the bryophytes, only liverworts have obligate mycorrhiza-like interactions, suggesting that liverworts and their mycorrhizal partners possibly play an essential role in the stabilization of disturbed habitats, especially in the humid tropics. The colonization process was monitored in relevés, the involved bryophytes were identified, and the importance of their mycorrhization was assessed. Concerning geology and altitude, two sites were chosen for the investigations. The plots along the path above the Rio Bombuscaro (1000–1050 m a.s.l.) east of Zamora were on deeply weathered granite soils. The plots on the Camino Canal near ECSF (1800–1900 m) were on soils with a phyllitic base rock.

All plots were 1×1 m, shaded and had an inclination of between 75° and 90°. The development of the vegetation was recorded four times at 4-month intervals. Five plots at the Camino Canal and seven plots in Bombuscaro had already some vegetation and were therefore termed "succession plots". Six plots, the "initial plots", were without any vegetation. In five of those which were adjacent to successional plots, the 5 cm thick topsoil layer was removed. Total plant cover, cover of bryophytes and cover of vascular plants were separately recorded and only bryophytes were identified to species level. Cover abundance was determined exactly if values were lower than 15%.Values exceeding 15% were recorded in 5% steps. The frequency of mycorrhization was assessed using three small samples from each liverwort species.

Expectedly, total plant cover and cover of bryophytes and vascular plants increased on all plots during the observation period (Table 27.1). Total cover reached at least 70% at the end of the investigation period. The succession plots had

Table 27.1 Development of cover abundance (%) from the first to the last relevé. Symbols indicate the increase in cover abundance: + weak increase (1–20%), ++ moderate increase (21–40%), +++ strong increase (>40%). *Index,* cover abundance of liverworts divided by that of mosses

Plot no.	Total		Bryophytes		Vascular plants		Index
Initial plots							
S9b	0 → 70	+++	0 → 45	++	0 → 40	++	5
S8b	0 → 70	+++	0 → 60	+++	0 → 20	+	7
S10b	0 → 80	+++	0 → 70	+++	0 → 15	+	13
S12b	0 → 80	+++	0 → 80	+++	0 → 10	+	6
S1	0 → 85	+++	0 → 85	+++	0 → 15	+	5
S13b	0 → 95	+++	0 → 90	+++	0 → 15	+	24
Succession plots							
S3	10 → 90	+++	10 → 80	+++	1 → 25	++	2
S2	15 → 80	+++	15 → 80	+++	2 → 10	+	6
S4	50 → 85	++	40 → 70	++	10 → 45	++	4
S6	55 → 90	++	40 → 65	+	15 → 70	+++	6
S5	60 → 80	+	50 → 65	+	15 → 40	++	10
S7	60 → 95	++	55 → 85	++	10 → 20	+	10
S8a	60 → 85	++	60 → 80	+	5 → 10	+	5
S10a	65 → 90	++	55 → 75	+	10 → 45	++	8
S9a	70 → 95	++	30 → 60	++	50 → 90	++	6
S13a	70 → 95	+	50 → 75	++	30 → 65	++	14
S12a	85 → 100	+	70 → 100	++	20 → 35	+	13
S11	90 → 100	+	75 → 90	+	20 → 40	+	8

a slightly higher average total cover (90%) than the initial plots (80%). Provided that conditions for growth of bryophytes were favorable (i.e. high air and substrate humidity, sufficient diaspore rain) the vegetation cover of some initial plots in Bombuscaro was nearly re-established within 9 months (Fig. 27.2). The composition of species, however, was far from constant after this period. At the end of the observation period, the bryophytes reached at least 60% cover on the succession plots and 45% on the initial plots. The range of abundance of the vascular plants was considerably wider (10–90%) than for the bryophytes (45–100%) and average values differed substantially between the succession plots (41%) and the initial plots (18%).

The succession on the investigated plots is biphasic. Phase 1 (the actual pioneer phase) is characterized by the dominance of bryophytes. All investigated plots with low initial total cover (0–20%) show a strong increase of bryophyte cover. Phase 2 (stabilization phase) is characterized by upcoming vascular plants. On plots with an initial total cover of more than 50%, the increase in the abundance of vascular plants sometimes exceeds that of the bryophytes. However, at the end of the observation period, bryophytes cover a significantly greater area than vascular plants.

A comparison of the three bryophyte groups shows that liverworts are most important. Cover abundance of the liverworts exceeds that of the mosses 2- to 24-fold. Hornworts are negligible. The common pioneer vegetation is dominated by only a few species, in our case thalloid liverworts: *Riccardia amazonica* is present in 89% of the

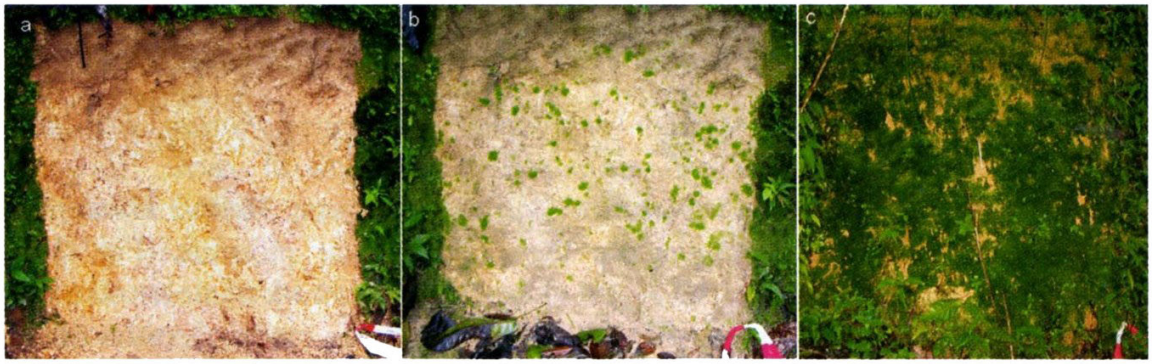

Fig. 27.2 Documentation of the (re-)colonization of plot S12b (see Table 27.1). Photographs were taken on 20 July 2004 (**a**), 05 October 2004 (**b**) and 21 April 2005 (**c**)

plots, frequently reaching the highest cover abundances. *Symphyogyna brongniartii* is present in 72% of the plots but hardly gets dominance. Members of the foliose genera *Calypogeia* and *Lophocolea* are present in many plots but at a low cover abundance. Among the mosses, only *Pogonatum tortile* plays an important role in some plots. Algae could be detected only on a few plots under relatively wet conditions.

Clear trends are visible concerning the mycorrhization of the liverworts. *Riccardia amazonica*, the most important species was never mycorrhized. Producing enormous amounts of generative and vegetative propagules, it was one of the very first colonizers in all initial plots forming dense patches within a few months. In a few plots its climax was already outrun within the observation period. *Paracromastigum bifidum*, another non-mycorrhized species, proved to be a fast and dominant colonizer in two plots in Bombuscaro. Liverwort species of a later successional stage were regularly mycorrhized. *Symphyogyna brongniartii* was the most frequent one, whose cover abundance increased steadily but never reached high values. This species is dependent on generative reproduction and apparently needs more time for colonization. Likewise *Marchantia chenopoda*, in spite of its ability for asexual reproduction, was encountered only on few plots, where it never reached dominance. The same holds for the foliose genera *Calypogeia*, *Cephalozia* and *Lophocolea*. A successional decrease in cover abundance of non-mycorrhized liverworts was balanced by an increase in species with fungal symbiosis. The type of the mycorrhizal partner seems to have little influence on the colonization process. Similar colonization rates were observed with Glomeromycetes (in *Symphyogyna* and *Marchantia*) or Ascomycetes (in *Calypogeia* and *Lophocolea*) as the fungal symbionts. Even young plants were already mycorrhized, indicating the strong dependence of these liverworts on their fungi. An interesting aspect is the quantity of rhizoids of the respective species. The non-mycorrhized species had only a few rhizoids while the mycorrhized species produced long and dense rhizoids. Together with proliferation of the mycelium, rhizoid formation contributes to the stabilization of the topsoil.

First experiments with homogenates of the above species show good results in initiating the re-colonization of steep patches of bare soil. Spraying such homogenates, liverworts could contribute to stop erosion in the area.

27.3.3 Colonization by Vascular Plants

Several species of orchids (*Epidendrum lacustre*, *E. carpophorum*, *Elleanthus aurantiacus*), a few bushes (*Escallonia paniculata*, *Gaultheria erecta*, *Tibouchina lepidota*), grasses (*Andropogon bicornis*, *Cortaderia jubata, Melinis minutiflora*) and the fern *Blechnum cordatum* are the first vascular pioneers (Fig. 27.3). *Elleanthus aurantiacus*, e.g. gets a foothold by a dense network of stolon-like roots with a *Velamen radicum*. The bushes and grasses colonize cracks, erosion ravines and small ledges where fine soil material accumulates.

Ferns, mainly of the genus *Sticherus*, cover the nearly vertical flanks of upper embarkments. *Sticherus rubiginosus* heavily propagates vegetatively by runners, forming a curtain-like cover (Ohl and Bussmann 2004).

27.3.4 Regeneration of a Vegetation Cover on Accumulated Soil Material

On less steep upper embankments, where some soil material has been left, recovery of the vegetation is much faster and several successional stages can be found along

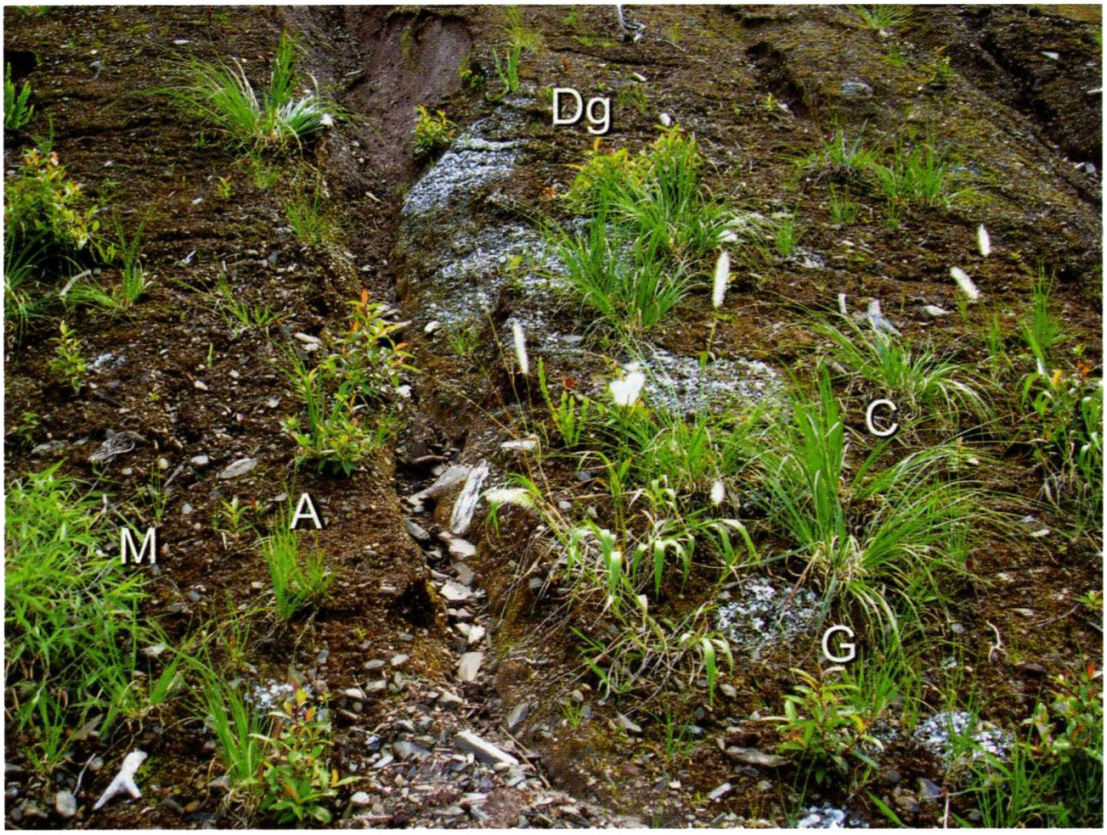

Fig. 27.3 Stabilization of an upper embankment by phanerogams, lichens and mosses/liverworts: *Andropogon bicornis* (*A*), *Cortaderia jubata* (*C*), *Melinis minutiflora* (*M*), *Dictyonema glabratum* (*Dg*), *Gaultheria erecta* (*G*)

a trail or road. Such stages differ from the plant cover of abandoned pastures by the absence of species (e.g. bracken) which are typical of areas where the forest has been cleared by fire.

Successional stages which are apparently stable for a longer time are:

1. A dense herbal vegetation with a high abundance of grasses and orchids (Table 27.2);
2. Two bush stages as potential transient stages to
3. Secondary forests.

The plant cover of these successional stages is not homogeneous and therefore several phenotypes may be differentiated by the dominant herbs and bushes.

Table 27.2 Characteristic compositions of the herbal stage. Several vegetation subtypes could be classified based on the dominant species and the species composition (from 1a to 7). Cover abundances according to the Braun–Blanquet scale

		Subtype										
Type	Species	1a	1b	2a	2b	3	4a	4b	4c	5	6	7
Mosses, lichens and herbs	*Baccharis genistelloides*	1	1	1	1	1	1	1	1	1	1	–
	Andropogon bicornis	2	1	2	1	2	3	3	3	–	–	1
	Andropogon leucostachys	3	3	2	3	–	–	–	–	2	2	–
	Cortaderia jubata	2	2	–	–	2	–	–	–	–	2	3
	Elleanthus aurantiacus	–	–	1	–	–	1	1	1	1	1	–
	Pteridium arachnoideum	–	–	1	–	–	–	–	–	–	–	–
	Sobralia fimbriata	–	–	1	–	–	–	–	–	–	–	–
	Sticherus rubiginosus	–	–	1	1	–	1	–	–	–	–	–
	Lycopodium sp.	–	1	–	–	1	1	–	–	–	–	–
	Epidendrum carpophorum	–	1	–	–	–	–	1	–	–	1	–
	Frullania sp.	–	2	–	3	–	–	–	–	–	–	–
	Epidendrum lacustre	–	1	–	1	–	–	–	–	–	1	–
	Sphaerophorus sp.	–	1	–	1	1	–	–	1	–	–	–
	Maxillaria aurea	–	–	–	–	–	–	–	1	–	–	–
	Melinis minutiflora	–	–	–	–	–	–	2	3	–	–	3
Bushes	*Bejaria aestuans*	–	–	2	–	–	–	–	–	–	–	–
	Escallonia paniculata	–	1	–	–	–	–	–	1	–	–	–
	Tibouchina lepidota	–	1	–	1	–	–	–	–	–	–	–
	Gaultheria erecta	–	–	–	1	1	–	–	1	–	–	–

27.3.4.1 The Herbal Stage

The dominant species of the herbal regeneration stage (Table 27.2) show xerophytic characters like small leaves (*Baccharis genistelloides*) or densely hirsute leaf surfaces (*Melinis minutiflora*) and exhibit a high potential of vegetative propagation, especially so orchids and grasses. The height of the plant cover commonly exceeds 50 cm, but rarely reaches 1 m. The few bushes may be interpreted as remnants of a former pioneer stage (e.g. *Escallonia paniculata*) or as forerunners of the bush stage (e.g. *Bejaria aestuans*).

27.3.4.2 The Bush Stage

The bush stage is more difficult to categorize and only early and late stages could be differentiated (Fig. 27.4). Twenty-five relevés were examined for consistency and cover abundance using the percent Londo scale (Londo 1976). In a space-for-time substitution (Pickett and White 1986) structural elements were used as indicators of succession. The dominance of grasses and herbs was considered as an indication of an early stage while the occurrence of pioneer tree species was symptomatic of a late successional stage. This graduation agreed fairly well with an index ("diversity index") calculated from the number of bush species multiplied by the sum of their cover abundances. Fourteen relevés could be attributed to the early stage (diversity index 75–290), while 11 represented the late phase (diversity index 420–1850). Fig. 27.4A shows the general decrease in the cover abundances of herbs and grasses from the early to the late stage, and Fig. 27.4B, C show the concomitant increase of bushes, lianas and trees. Pioneer bushes, e.g. *Monochaetum lineatum* and *Gaultheria erecta*, like herbs and grasses, decreased in cover abundance as the succession progressed. *Myrica pubescens* and *Baccharis latifolia*, which do not exhibit a correlation with successional stages, can be addressed as general indicators of disturbance. Both species are frequent on those lower embankments, where secondary forest cannot establish, due to an ongoing disturbance or an unfavorable soil.

The successional bush stage, in contrast to the climax on abandoned agricultural areas, leads to a secondary forest if the disturbance is not permanent and the area borders the natural forest.

27.3.4.3 Secondary Forest

Various secondary forests could be differentiated, which at least partly can also be interpreted as space-for-time series. As secondary forests are not only found as forest regeneration stages following the bush stage, but also as remnants of fire-attacked primary forest, they are treated separately (see Chapter 32 in this volume).

Maintenance of a road or a power supply line as vital lines requires an ongoing mechanical removal of the overgrowing vegetation and therefore the composition of the plant cover remains the same over the time, varying at most with the seasons.

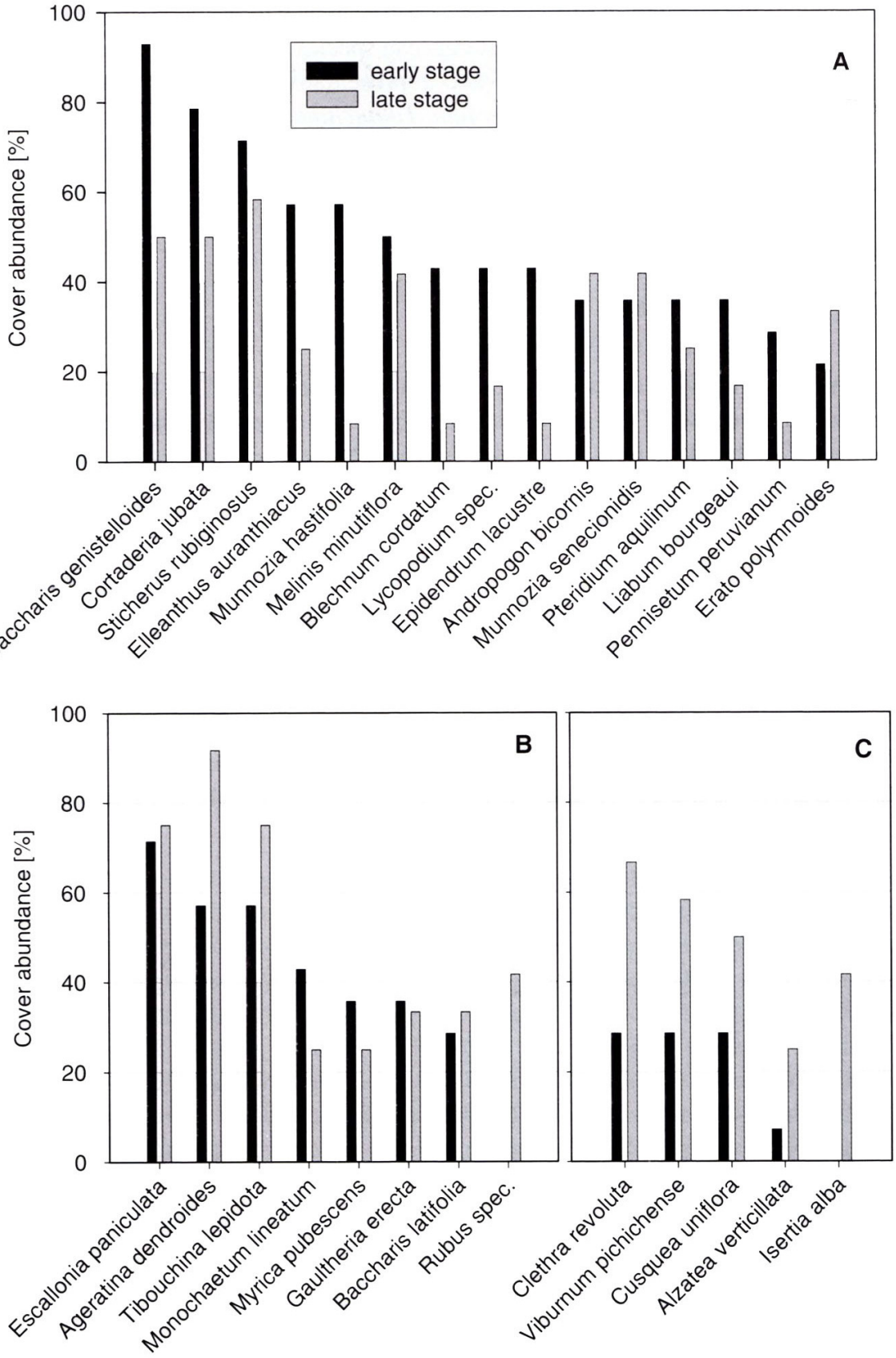

Fig. 27.4 Changes in the species composition from the early to the late bush successional stage. **A** Decrease in the abundance of herbs. **B** Overall increase in bush cover. **C** Increase in the cover abundances of trees and *Chusquea* sp., the typical liana of secondary forests. Only those species are shown with cover abundance >10%, which was not reached by another 34 herbal, 11 bush and 14 tree species (from Hartig and Beck 2000)

This type of the vegetation forms a reservoir for seeds which spread to cleared areas in the vicinity, e.g. to the pastures. Asteraceean species like *Baccharis genistelloides*, *B. latifolia*, *Ageratina dendroides* and representatives of the Melastomataceae (*Brachyotum* sp., *Monochaetum lineatum*, *Tibouchina lepidota*) are typical components of the wayside vegetation which are also major constituents of the mixed bracken and bush vegetation of the abandoned pastures.

27.4 Conclusions

In contrast to the wide areas where the forest has been cleared for farming purposes and which (because of the use of fire) finally develop a kind of climax vegetation composed of bracken fern and bushes, forest can recover alongside linear disturbances, if the disturbance is not maintained. Several successional stages could be differentiated, starting with a cryptogamic stage, characterized by liverworts, lichens and mosses. Although colonization of suitable bare ground especially by liverworts is extremely fast, on bedrock or otherwise sealed surfaces the cryptogamic stage may last for decades until sufficient soil material accumulates to allow growth of a closed plant cover which can re-develop into a secondary forest. Continuation of the disturbance by human maintenance activities results in a wayside vegetation which heavily exchanges propagules with the adjacent open areas. Rigorous road construction in steep terrain, however, has caused sizeable lesions in the vegetation which never recover and result in a permanent landslide activity.

Model parameterization to simulate the PAR absorption potential of two competing plant species

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ORIGINAL PAPER

Model parameterization to simulate and compare the PAR absorption potential of two competing plant species

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Abstract Mountain pastures dominated by the pasture grass *Setaria sphacelata* in the Andes of southern Ecuador are heavily infested by southern bracken (*Pteridium arachnoideum*), a major problem for pasture management. Field observations suggest that bracken might outcompete the grass due to its competitive strength with regard to the absorption of photosynthetically active radiation (PAR). To understand the PAR absorption potential of both species, the aims of the current paper are to (1) parameterize a radiation scheme of a two-big-leaf model by deriving structural (LAI, leaf angle parameter) and optical (leaf albedo, transmittance) plant traits for average individuals from field surveys, (2) to initialize the properly parameterized radiation scheme with realistic global irradiation conditions of the Rio San Francisco Valley in the Andes of southern Ecuador, and (3) to compare the PAR absorption capabilities of both species under typical local weather conditions. Field data show that bracken reveals a slightly higher average leaf area index (LAI) and more horizontally oriented leaves in comparison to *Setaria*. Spectrometer measurements reveal that bracken and *Setaria* are characterized by a similar average leaf absorptance. Simulations with the average diurnal course of incoming solar radiation (1998–2005) and the mean leaf–sun geometry reveal that PAR absorption is fairly equal for both species. However, the comparison of typical clear and overcast days show that two parameters, (1) the relation of incoming diffuse and direct irradiance, and (2) the leaf–sun geometry play a major role for PAR absorption in the two-big-leaf approach: Under cloudy sky conditions (mainly diffuse irradiance), PAR absorption is slightly higher for *Setaria* while under clear sky conditions (mainly direct irradiance), the average bracken individual is characterized by a higher PAR absorption potential. (~74 MJ m^{-2} $year^{-1}$). The latter situation which occurs if the maximum daily irradiance exceeds 615 W m^{-2} is mainly due to the nearly orthogonal incidence of the direct solar beam onto the horizontally oriented frond area which implies a high amount of direct PAR absorption during the noon maximum of direct irradiance. Such situations of solar irradiance favoring a higher PAR absorptance of bracken occur in ~36% of the observation period (1998–2005). By considering the annual course of PAR irradiance in the San Francisco Valley, the clear advantage of bracken on clear days (36% of all days) is completely compensated by the slight but more frequent advantage of *Setaria* under overcast conditions (64% of all days). This means that neither bracken nor *Setaria* show a distinct advantage in PAR absorption capability under the current climatic conditions of the study area.

Keywords Southern bracken · *Setaria sphacelata* · PAR absorption · Two-big-leaf approach · Andes · Ecuador

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Introduction

The Ecuadorian Andes are one of the major hot spots of vascular plant diversity worldwide (Barthlott et al. 2007).

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At the same time, the natural forests in Ecuador suffer from the highest deforestation rate (1.2% per year) in Latin America (FAO 2001). In the eastern Cordillera, large areas of tropical forest have been cleared by slash and burn for gaining pastureland. On slopes of moderate inclination, the *Sorghum*-like tillering *Setaria sphacelata* is grown in monocultures. Our previous studies on *Setaria* pastures have shown that the current mode of pasture management favors the growth of an extremely aggressive weed, the tropical bracken fern *Pteridium arachnoideum* (Hartig and Beck 2003). This process is particularly prominent in the lower parts of the Rio San Francisco valley <2,400 m asl (Fig. 1) where large areas (~68%) of former pastures have been abandoned due to bracken infestation and the concomitant supersession of the pasture grass (Beck et al. 2008a; Göttlicher et al. 2009). Unfortunately, the loss of pasture usability boosts the land use pressure on the remaining natural forest, threatening its unique biodiversity.

Consequently, bracken should be effectively controlled to retain pasture productivity, but to date, the reason for its competitive strength is not well understood. Some investigations suggest that spreading and growth of bracken seems to be stimulated by burning (e.g., Page 1986; Cruz et al. 2009). Other studies on bracken in England and Mexico (Marrs et al. 2000a, b; Schneider 2004) point out that bracken fronds cast shadow on underlying plants, thus outcompeting understorey vegetation like grass tufts. Field observations in the study area show that bracken accelerates growth after burning which suggests that the shading of the pasture grass by the fast emerging fronds seems to boost the dominance of bracken after recurrent burning increasingly (Hartig and Beck 2003). However, final evidence is still lacking. Numerical simulation models encompassing radiative transfer approaches (e.g., Pronk et al. 2007) might help to unveil the importance of frond shadow for the competitive strength of bracken in the study area. For spatial

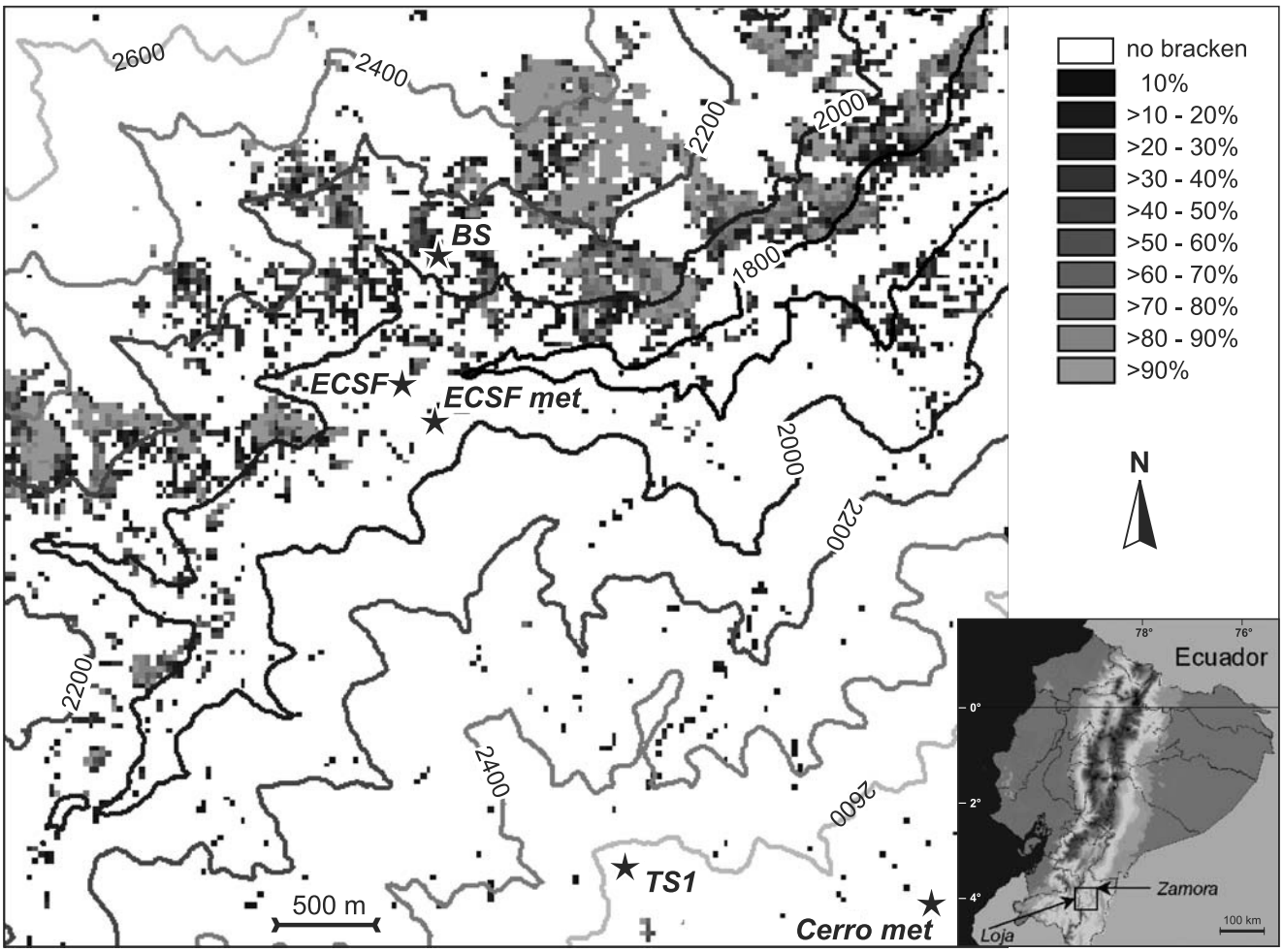

Fig. 1 The research area showing fractional cover by southern bracken as derived from Landsat TM data with the probability guided spectral unmixing technique (Göttlicher et al. 2009); *BS* experimental bracken site and micrometeorological station, *ECSF* Estación Científica San Francisco, *ECSF met* Meteorological station of the Estación, *TS1* and *Cerro met* are meteorological stations located at 2,660 and 3,180 m asl, respectively. *Grey shades* indicate different bracken coverage per pixel, *white* means bracken-free pixel

applications, grid-cell based two-big-leaf models have been proven to be computationally efficient (Wang and Leuning 1998) with good accuracy compared to more complex multi-layer models (Zhang et al. 2001). The first research question in order to estimate the competitive strength of species with regard to radiation available for plant growth is if the two competing species reveal different PAR (photosynthetically active radiation) absorption potentials.

Consequently, the main objective of the current paper is to compare the PAR absorption potential of the two competing plant species, *Setaria sphacelata* and *Pteridium arachnoideum*, as the basis for future research on growth competition modeling using a numerical vegetation growth model. The comparison is conducted by applying the radiation scheme of a state of the art grid-based two-big-leaf model (Dai et al. 2004; Thornton and Zimmermann 2007). Because the model is normally operated with default parameters for broad groups of plant functional types (broadleaf trees, grass, etc.), a proper parameterization with structural and optical plant traits is required to adapt the scheme to the competing species (e.g., Larocque 2002; Wang et al. 2006b; Boulain et al. 2007). With regard to structural plant traits, Lappi and Stenberg (1998), for instance, stressed that simulation of PAR interception/absorption is highly dependent on the relation of leaf orientation and solar geometry.

Thus, the second goal of the current study is to adapt the radiation scheme to the two species of interest by providing the required structural and optical plant traits based on extensive field surveys. The properly parameterized scheme is then used to simulate the PAR absorption capabilities of both species under the varying and realistic illumination conditions of the study area in the Andes of southern Ecuador.

Materials and methods

Study area and data

The current investigation is part of a multidisciplinary ecological research project. The study area comprises parts of the deeply incised valley of the Rio San Francisco in the eastern range of the South Ecuadorian Andes in the vicinity of the research station Estación Científica San Francisco (ECSF, 3°58′18″S, 79°4′45″W, alt. 1,860 m asl; Fig. 1). The station is situated between the provincial capitals of Loja in the inner-Andean basin west of the main cordillera and Zamora in the foothills of the eastern Andes. The core area displayed in Fig. 1 encompasses an altitudinal gradient from 1,800 to 3,200 m asl. A detailed description is given in Bendix et al. (2006a) and Beck et al. (2008b). The climate of the Rio San Francisco valley is perhumid (Richter 2003; Bendix et al. 2008a, b). Important for the radiation conditions is the high cloud frequency over the entire year, especially at higher altitudes (Bendix et al. 2006b, 2008c). While the north-facing slopes of the valley are covered by a species-rich natural mountain forest (Brehm et al. 2008), the south-facing slopes have been cleared by slash and burn for the acquisition of pasture land where large portions of the lower terrain between 1,800 and 2,500 m are already infested or completely overgrown by bracken (Fig. 1).

Long-term meteorological data for the current study were available from the ECSF meteorological station located at 1,960 m asl (ECSF met in Fig. 1), the altitudinal level of the bracken-infested pastures. At this station, global radiation has been measured since 1998. Monthly means of global radiation from 1998–2005 as presented in Bendix et al. (2008a) were used in this study. Additionally, an experimental site (BS in Fig. 1) consisting of ten 10×10 m plots with different fractions of bracken and *Setaria* was established in 2007 in the vicinity of the main meteorological station (ECSF met). The site encompasses a micrometeorological station that measures global radiation at 5-min temporal resolution. Global radiation at both stations was measured with the Kipp & Zonen CM3 pyranometer for the entire solar spectrum (spectral range 305–2,800 nm).

Radiation scheme

To calculate PAR absorption by bracken and *Setaria*, the radiation scheme of the two-big-leaf approach of Dai et al. (2004) was applied. Generally, big-leaf approaches describe the water and gas exchange of vegetation in a simple way where the canopy is treated as one layer with a single physiological and aerodynamic resistance to water /CO_2 transfer. A big-leaf model generally encompasses (1) a radiation scheme as discussed in this study, (2) a leaf model accounting for the interaction of conductance and photosynthesis and the response of stomata to water vapor pressure deficit and available soil water, and (3) a parameterization of radiative conductance to solute the leaf energy balance equation (Wang and Leuning 1998). The two-big-leaf extension separates the whole canopy leaf area into sunlit and shaded leaf portions and the canopy-average PAR values are estimated for each leaf portion, which needs the application of radiative transfer calculations between the sunlit and shaded leaf fraction and the underlying soil (Zhang et al. 2001). Species are represented in a two-big-leaf models as a single plant with one sunlit and one shaded leaf. Average traits (e.g., leaf albedo) that are representative for the species are assigned to the model plant.

It is presupposed that the sunlit leaf receives both diffuse and direct radiation while the shaded leaf receives diffuse light only.

The photon transport among the atmosphere, the sunlit and shaded leaf area, and the ground is calculated by using the two-stream approximation with single scattering and uniform leaf orientation as presented in Dickinson (1983) and Sellers (1985). This module is the central part of the radiation scheme of Dai et al. (2004) which was used in the current study. With regard to canopy albedo, Myneni et al. (1992) showed that the accuracy of similar 1D radiative transfer schemes is adequate in comparison to more complex and computational expensive 3D approaches. Also, the prediction of PAR provided reasonable values when compared with a more complex multi-layer model (Zhang et al. 2001).

The numerical solutions and all relevant equations of the radiation scheme used in this study are already published in Dai et al. (2004) and will not be repeated here in detail.

In the current study, the scheme is initialized by global radiation data at hand so that the decomposition for the direct, diffuse and PAR fraction is necessary.

On the plant level, the study focused on an average individual of bracken and the pasture grass *Setaria*. PAR absorption of the two species is strongly dependent on their functional traits that have to be derived from field observations to parameterize the radiation scheme properly. The first plant trait (or model parameter) of importance is the average leaf angle because it determines the leaf orientation to the sun and thus radiation absorption. In the radiation scheme, the average leaf projection $G(\mu)$ is used which is derived from (Dai et al. 2004):

$$G(\mu) = \phi_1 + \phi_2 \cdot \mu; \qquad \phi_1 = \left(0.5 - 0.633 \cdot \chi - 0.33 \cdot \chi^2\right); \qquad \phi_2 = 0.877 \cdot (1 - 2 \cdot \phi_1) \tag{1}$$

where μ is the cosine of the solar zenith angle, ϕ_1 and ϕ_2 are coefficients from the Ross-Goudriaan function (see Sellers 1985) and χ represents the leaf angle distribution (1= horizontal, −1=vertical, 0=spherical leaf angle distribution) that have to be measured for representative individuals of bracken and *Setaria* in the experimental plots. It is obvious that the leaf orientation in relation to the solar angle determines drop shadow on shaded leaves and thus, the sunlit and shaded fractions of leaf area (see Dai et al. 2004).

The second important trait is the spectral leaf albedo because it determines the proportion of solar radiation remaining available for absorption and transmission. For instance, the direct incident beam radiation absorbed by leaves at canopy depth × per unit leaf area index L (excluding scattering) is calculated in the scheme as (Dai et al. 2004):

$$I_{lb} = (1 - \omega) \cdot kb \cdot \exp(-kb \cdot xL) \cdot I_{b0} \tag{2}$$

where I_{b0} is the incident direct beam radiation above the canopy, kb the direct beam extinction (see Dai et al. 2004) and the leaf scattering coefficient ω is:

$$\omega = \alpha + \tau \tag{3}$$

with α is the spectral leaf albedo (second parameter) and τ the spectral leaf transmittance as the third important functional trait, determining the transmission of radiation to the shaded leaf area fraction and the underlying bare soil. Consequently, both optical parameters have to be derived from field measurements of representative individuals of bracken and *Setaria*. Equation (2) also uses the leaf area index that is available for PAR absorption. Thus, also the average leaf area index is a model parameter that has to be derived from field observations.

On the soil level, soil spectral albedo has to be measured because it determines the backscattered direct and diffuse radiation fractions in direction of the green phytoelements which are generally available for PAR absorption, increasing with soil albedo (Nouvellon et al. 2000).

Decomposition of global radiation data

The two-big-leaf scheme requires the diffuse and direct fractions of solar radiation as input. Because only global radiation was measured at the meteorological stations, we used an empirical decomposition function to partition incident solar radiation in its diffuse and direct fractions. Generally, such functions are based on the diffuse fraction of radiation (k') and the clearness index (kt). The clearness index (kt) is the ratio of incoming radiation at the earth surface (I_0) to the radiation at the top of the atmosphere on a horizontal surface (I_{TOA}).

$$kt = I_0 / I_{TOA} \tag{4}$$

Diffuse I_{d0} and direct I_{b0} radiation incident above the canopy is then derived by:

$$I_{d0} = I_0 \cdot k' \tag{5}$$

$$I_{b0} = I_0 \cdot (1 - k') \tag{6}$$

where I_0 is the incident global radiation above the canopy.

Empirical radiation decomposition functions are generally more or less site dependent. To find the most appropriate function for the San Francisco valley, six published functions are examined. Erbs et al. (1982) used data sets from U.S.

locations to formulate a piecewise regression function that has been widely and successfully used in other parts of the world (Elminir 2007). A modified version of Erbs function was recently proposed by Wang et al. (2006a). Two versions of a function of Boland et al. (2001, 2008) for Australia yields lower fractions of diffuse radiation at a high clearness index, which could principally apply to situations in high elevations in the absence of clouds. A function taking annual seasonality into account was determined for southeast Brazil by Oliveira et al. (2002). Maduekwe and Chendo (1994) presented a numerical solution with solar elevation as a second predictor for the diffuse fraction. Figure 2a shows the long-term averaged (1998–2005) daily course of diffuse radiation calculated from the long-term incident global radiation at the ECFS meteorological station. The diffuse fraction as a function of the clearness index is also displayed (Fig. 2b). The data show that the fraction of diffuse radiation in the study area is generally high. In the early morning and late afternoon hours, almost the only radiation present is diffuse, while around noon, direct irradiance accounts for approximately one third . The dominance of diffuse radiation is mainly due to the overall high cloudiness of ~80% over the day and the year in the San Francisco valley (Bendix et al. 2006b, 2008c).

The function of Boland et al. (2001) (BSL), with the solar apparent time as predictor (BSLa at 0800 and BSLb at 1700 hours in Fig. 2b), results in relatively strong deviations in the afternoon. Oliveira′s function (OESM), which summarizes the winter (OESMa) and summer (OESMb) regressions, gives the lowest diffuse radiation fraction. The other four decomposition functions which comprise Boland et al. (2008) (BRB) and Erbs et al. (1982) (EKD) give more or less identical daily courses. The difference between the mean of these functions and Oliveira′s solution varies between −1 and +22% of the global radiation. For the current study, we used the modified Erbs′ function (EKD2) because it considers the increase of diffuse radiation on lower solar elevation (see Fig. 2b where EKD2 is calculated for a solar elevation of 15°). The diffuse fraction k' is calculated in a two-step procedure as follows:

$$k = \begin{cases} 1 - 0.09 \cdot kt & 0 \le kt \le 0.22 \\ 0.9511 - 0.1604 \cdot kt + 4.388 \cdot kt^2 - 16.638 \cdot kt^3 + 12.336 \cdot kt^4 & 0.22 < kt \le 0.8 \\ 0.165 & 0.8 \le kt \le 1. \end{cases} \quad (7)$$

$$k' = \frac{k}{(1-k) \cdot \mu + k} \quad (8)$$

where k' is the diffuse fraction of radiation modified due to solar geometry with the cosine of solar zenith angle μ.

To obtain the PAR fraction from global radiation, a conversion factor of 0.48 is generally multiplied by I_0, but Zhang et al. (2000) and Lee and Hernández-Andrés (2005) showed that the PAR fraction varies particularly in the presence of clouds. A slight increase of the PAR fraction from clear to cloudy sky due to larger forward scattering was observed by Papaioannou et al. (1993) and Roderick et al. (2001). Tsubo and Walker (2005) considered clouds by applying the clearness index as predictor for the PAR fraction. Consequently, this function addresses best the high cloud frequency in the San Francisco valley and is therefore used in the current study:

$$I_{PAR} = \left(0.121 \cdot kt^2 - 0.334 \cdot kt + 0.613\right) \cdot I_0 \quad (9)$$

Field observations of plant and soil parameters

The two required structural plant parameters, the leaf area index and the leaf to ground angles, were measured with a LICOR LAI-2000 plant canopy analyzer. Leaf area index and leaf angles were taken for representative *Setaria* and bracken individuals. A total of 24 measurements for each species were conducted between October 2007 and March 2008 on the experimental site (BS in Fig. 1). Canopy leaf angle was determined as mean tilt angle (MTA) (see Peri et al. 2003) that, however, is in good agreement with directly derived mean leaf angles (Antunes et al. 2001). Mean tilt angle orientation of measured foliage is converted to the leaf angle parameter χ (Eq. 1) by:

$$\chi = \cos(MTA) - \sin(MTA) \quad (10)$$

The two optical plant traits (leaf albedo and transmission) and soil albedo were measured with the field spectrometer Tec5 HandySpec Field 14 during late 2007 and early 2008. The instrument encompasses a Zeiss MMS 1 NIR enhanced (310–1,100 nm, dλ=3.3 nm) and a Zeiss Plangitter PGS NIR 1.7 (960–1,690 nm, dλ=1.5 nm) sensor. For reflection measurements, fresh leaves of representative individuals were collected and inserted in a wooden frame to normalize the measurements to a leaf area index of 1. Average values were calculated from three measurements of each species. Topsoil samples were taken from the experimental bracken site, representing the soil under bracken and grass. Spectral integration of the data was conducted for PAR (<700 nm) and NIR (≥700 nm).

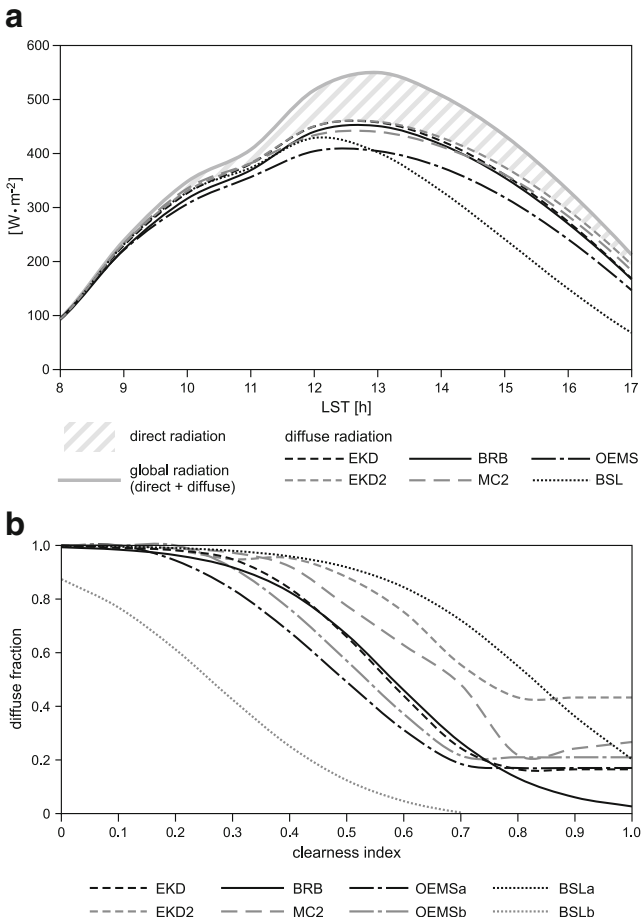

Fig. 2 **a** Average diurnal course of global and derived diffuse radiation at ECSF met station (1998–2005). **b** The diffuse fraction from six decomposition functions is also shown as a function of the clearness index (*bottom*). For abbreviations, see text

Results

Derived plant and soil traits

The observed values of LAI and χ for representative individuals of *Setaria* and bracken are presented in Table 1. They show that LAI and χ (indicated by the mean tilt angle) differ between the samples and thus, the observation situation. However, the principle of the two-big-leaf approach only permits one average individual per species. Thus, the plants selected in the field survey which are presented in Table 1 are chosen to properly represent an average individual. The calculated average structural plant traits are then assigned to the average *Setaria* and bracken individual used by the radiation scheme which is applied in the next subsection. The same holds for the optical traits presented in Table 2.

With regard to the field data of structural plant traits presented in Table 1, the average leaf are index of bracken (2.94) is higher than that of *Setaria* (2.4). The standard deviation reveals that the LAI of bracken is characterized by a slightly higher variation in comparison to *Setaria*.

Additionally, Table 1 points to a clear difference in leaf angle orientation towards more vertical leaves for the *Setaria* tufts and more horizontally oriented leaf blades for bracken where the span of leaf angles is generally higher for bracken than for *Setaria*. The grass reveals a mean leaf angle of ~59° and an average leaf angle distribution parameter of $\chi = -0.37$ which clearly confirms the more vertical orientation of the grass stalks. MTA values and the low standard deviation show that the angular distribution of *Setaria* culms is almost unimodal. With regard to bracken, MTA seems to points to a bimodal distribution. Bracken fronds are composed of a long vertical petiole and a nearly triangular tripinnate lamina. Emerging fronds, whose lamina are still unfolded, have not been included in the study. Unfolding of the leaf blade starts when it turns from a vertical position into an angle between 40 and 50°. During further unfolding of the lamina, the leaf blade approaches a more horizontal orientation (MTA of 0–8°). Because an average fern canopy consists of young and mature leaves, the average individual of the two-big-leaf approach must consider both representations which is warranted by the mean leaf angle parameter of $\chi = 0.48$.

The albedo data gathered during our field surveys show that both plant species exhibit a marked red edge spectrum of green vegetation with a slightly enhanced reflectance in the green waveband (Fig. 3).

In contrast, the topsoil reveals the typical, almost linear increase of reflectance towards the near infrared. Bracken contrasts with *Setaria* by a lower reflectance in the PAR and a higher reflectance in the NIR. Transmittance of bracken fronds is slightly higher than that of *Setaria* over the whole spectrum. The integrated optical traits for the PAR spectral range (300–700 nm) as derived from the measurements of the field spectrometer for both species (*Setaria* and bracken) are presented in Table 2. Average integrated PAR reflectance of *Setaria* (11.7%) is higher than bracken PAR albedo (7.4%). At the same time, integrated PAR transmittance of *Setaria* (1.5%) is lower in comparison to bracken (3.7%), resulting in a slightly higher absorptance of 2.1% for bracken which might potentially favor bracken with regard to photosynthesis.

Simulated PAR absorption capability

The first quantity that determines the PAR absorption capacity is the species-specific leaf–sun geometry which is mainly influenced by the average leaf angle parameter from Table 1. Figure 4 demonstrates the changing illumination situation for the average individuals of *Setaria* and bracken depending on solar elevation in relation to leaf

Table 1 Leaf area index (*LAI*) and mean tilt angle (*MTA*) of 24 representative measurements of *Setaria sphacelata* and bracken (*Pteridium arachnoideum*) on the experimental site

Sample	*Setaria*		Bracken	
	LAI (m^2 m^{-2})	MTA (deg.)	LAI (m^2 m^{-2})	MTA (deg.)
1	1.31	58	1.38	8
2	1.79	73	1.51	40
3	1.32	62	1.65	49
4	1.75	60	1.41	0
5	1.38	61	1.88	8
6	1.76	54	1.40	31
7	1.99	57	2.17	28
8	3.84	45	2.24	46
9	2.42	59	2.87	0
10	3.20	59	3.21	36
11	2.76	42	2.85	0
12	3.95	45	4.76	40
13	1.54	76	3.53	40
14	1.50	76	4.08	42
15	2.42	63	2.23	40
16	3.00	65	2.60	0
17	2.71	59	5.27	41
18	2.04	62	3.97	40
19	1.52	75	2.65	13
20	1.72	63	3.27	37
21	4.34	51	4.88	0
22	3.41	57	3.95	36
23	2.76	60	3.24	0
24	3.11	53	3.50	0
Average	2.40	59.79	2.94	23.96
1σ	0.90	9.14	1.15	18.87
Average χ		-0.37		0.48

orientation. The sunlit and shaded leaf fractions of bracken with its more horizontally oriented fronds run inversely to *Setaria* with its more upright leaves. At higher solar elevations (>28°) around noon (i.e., at solar zenith lower than 62°) bracken shows a smaller sunlit leaf fraction in comparison to *Setaria* because the sunlit bracken frond casts shadow on the underlying frond area due to the horizontal frond orientation. In contrast, shadowing around noon is clearly reduced in case of the vertically oriented leaves of *Setaria*. The situation changes in times of lower sun elevation (morning, evening) when cast shadow is preferentially caused by vertically oriented leaves. Then, bracken is favored in receiving direct radiation.

However, the sunlit and shaded leaf fraction must be scaled by the species-specific leaf area index (Table 1) of the average individuals to complete the view on leaf–sun geometry effects on PAR absorption potential. By doing so, *Setaria* sunlit leaf area exceeds that of bracken at solar zenith angles <55° (Fig. 5). The shift of the inversion point of the sunlit leaf fraction (see Fig. 4: 62° solar zenith angle) is an effect of the slightly greater LAI of the average bracken individual. This also explains the fact that the shaded leaf area of bracken exceeds the shaded leaf area of *Setaria* at all solar elevations.

Summarizing Figs. 4 and 5 could suggest that *Setaria* receives more direct radiation around noon in comparison to bracken. However, the amount of PAR absorption is not

Table 2 Integrated optical traits (*PAR*) of *Setaria sphacelata*, bracken (*Pteridium arachnoideum*) and bare soil. (Source: field observations with Handy-Spec)

	Setaria	Bracken	Bare soil
Reflectance (%) PAR (400 – 700 nm)	11.7	7.4	10.7
Transmittance (%) PAR (400 – 700 nm)	1.5	3.7	–
Absorptance (%) PAR (400 – 700 nm)	86.8	88.9	89.3

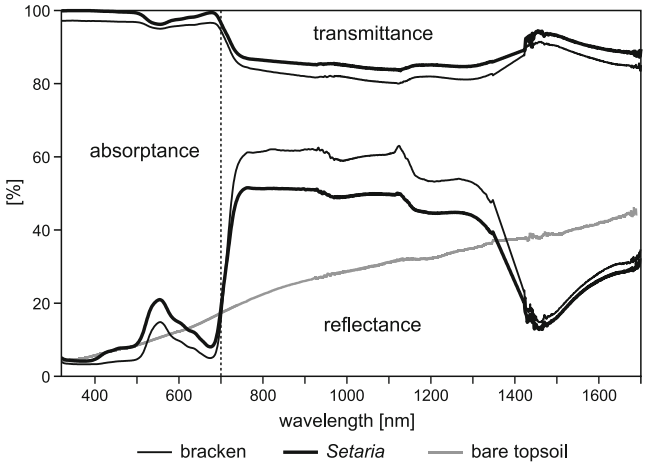

Fig. 3 Partitioning into reflectance (albedo), transmittance and absorptance of spectral radiation incident on *Setaria sphacelata*, *Pteridium arachnoideum* (as an average of three representative samples, LAI=1) and bare soil. The *traced line* represents the division between visible (PAR) and near infra red (>700 nm)

only a function of sunlit and shaded leaf area but is strongly dependent on (1) the resulting angle of incidence on the leaves for direct beam radiation, (2) the relation of direct/diffuse irradiance at the top of the canopy, and (3) the optical leaf traits. With regard to (1), a more perpendicular angle of incidence would be reached at low sun elevations for *Setaria* with its vertically oriented leaves when the sunlit leaf area is clearly reduced. In contrast, bracken shows the highest sunlit leaf area around noon when the solar rays are shining nearly perpendicular onto the horizontally oriented canopy fronds.

To address all governing factors (1–3) under realistic environmental conditions of the Rio San Francisco valley, a simulation was conducted which was initialized with the long-term averaged diurnal course of global irradiance (1998–2005) at the ECSF meteorological station (Fig. 1). The decomposition of global radiation was conducted by applying Eqs. 4–9. The results depicted in Fig. 6b clearly reveal that PAR absorption of bracken (4.2 MJ $m^{-2}day^{-1}$) is fairly equal to PAR absorption of *Setaria* (4.5 MJ $m^{-2}day^{-1}$.

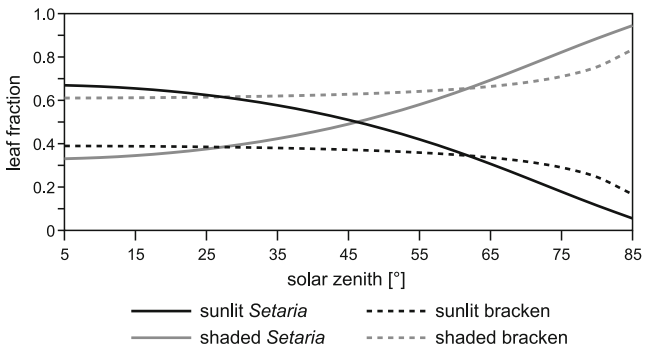

Fig. 4 Sunlit and shaded leaf fractions of bracken and *Setaria* for solar elevations between 5 and 85° (solar elevation=90°-solar zenith)

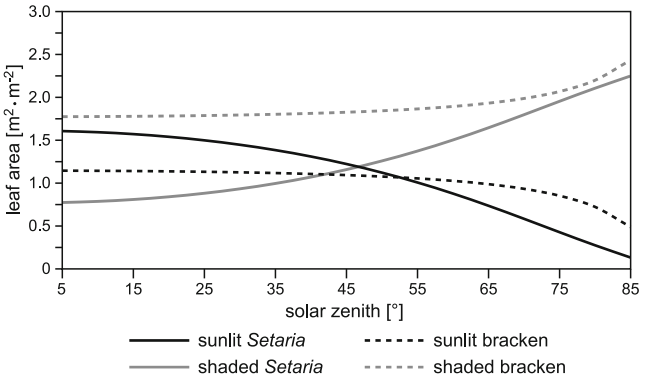

Fig. 5 Leaf area index ($m^2 \cdot m^{-2}$) of sunlit and shaded leaves of bracken and *Setaria* as depending on the solar zenith angle (solar elevation=90°-solar zenith)

Integrated over the day, the difference *(*ΔPAR) amounts to just 0.3 MJ $m^{-2}day^{-1}$. At first glance, this result is unexpected because considerable differences in sunlit and shaded leaf area of the two species exist (Fig. 6a).

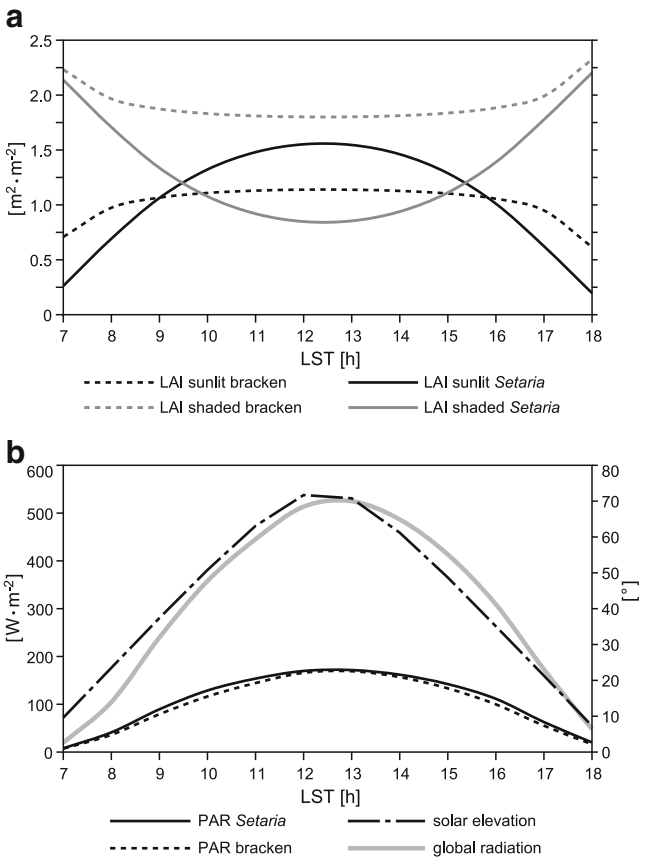

Fig. 6 **a** Leaf area indices ($m^2 \cdot m^{-2}$) of sunlit and shaded portions of the leaves of bracken and *Setaria* in the course of a day with regard to the average leaf–sun geometry of the study area. **b** Annual average of the diurnal course of irradiance at the ECSF meteorological station (1998–2005; Bendix et al. 2008a), sum of absorbed direct and diffuse PAR by sunlit and shaded leaves of bracken and *Setaria* plotted on the *left ordinate axis* and typical solar elevation in degrees on the *right ordinate axis*

This effect is most likely the result of the predominantly diffuse solar radiation available in the San Francisco valley due to the high cloudiness. The reason is that the available direct radiation at an inclined surface (leaf), following Lambert's law, depends on the incident angle of the light beam as a combination of leaf angle and position of the sun, but this angular dependence is not applicable to isotropically distributed diffuse radiation. Consequently, the species-specific differences in sunlit and shaded leaf area are not as crucial as they should be under clear sky conditions. As a result, bracken can compensate for the lower fraction of sunlit frond area by its slightly higher absorption coefficient and the total LAI exceeding that of *Setaria* (Tables 1 and 2). Thus, the total radiation surplus of *Setaria* yields not more than 0.3 MJ m^{-2} day^{-1} (4.2 MJ m^{-2} day^{-1} bracken, 4.5 MJ m^{-2} day^{-1} *Setaria*) in comparison to bracken over an average day in the Rio San Francisco valley.

To unravel the role of diffuse radiation on the PAR absorption potential, simulations were conducted for a typical sunny (5 December 2007) and cloudy (5 January 2008) day (Fig. 7).

The cloudy day with dominating diffuse irradiance reveals that *Setaria* has a slightly higher PAR absorption over the day (2.1 MJ m^{-2} day^{-1}) that exceeds that of bracken by 9.5% (or 0.2 MJ m^{-2} day^{-1}) (Fig. 7, right). However, it is striking that this potential growth advantage of *Setaria* is abolished during a sunny day with a great portion of direct solar irradiance around noon (Fig. 7, left). On such a day, bracken absorbs 8.5 MJ m^{-2} day^{-1}, which is 16.4% (or 1.2 MJ m^{-2} day^{-1}) more than total PAR absorption by *Setaria*. The reason of this inversion is illustrated in Fig. 8.

On sunny days, the major gain of PAR absorption by bracken as compared to *Setaria* is achieved by the absorption of incident direct beam radiation (I_0 *direct*) only. Here, the geometric configuration mentioned earlier takes effect. Fairly perpendicular incidence of the direct solar beam around noon (sun elevations 60–80°) onto nearly horizontally oriented bracken fronds provides the markedly higher direct radiation gain as compared to the nearly vertically oriented *Setaria* leaves. Even if PAR absorption of diffuse solar radiation (I_{d0}) and scattered diffuse radiation (I_{dif}) is higher for *Setaria* on the sunny day, this cannot compensate for the higher gain of direct beam PAR absorption by bracken fronds.

To test the PAR absorptance for different sunny and cloudy weather situations potentially occurring in the San Francisco valley, the ideal relative diurnal course of irradiance as measured on 5 December 2007 was taken

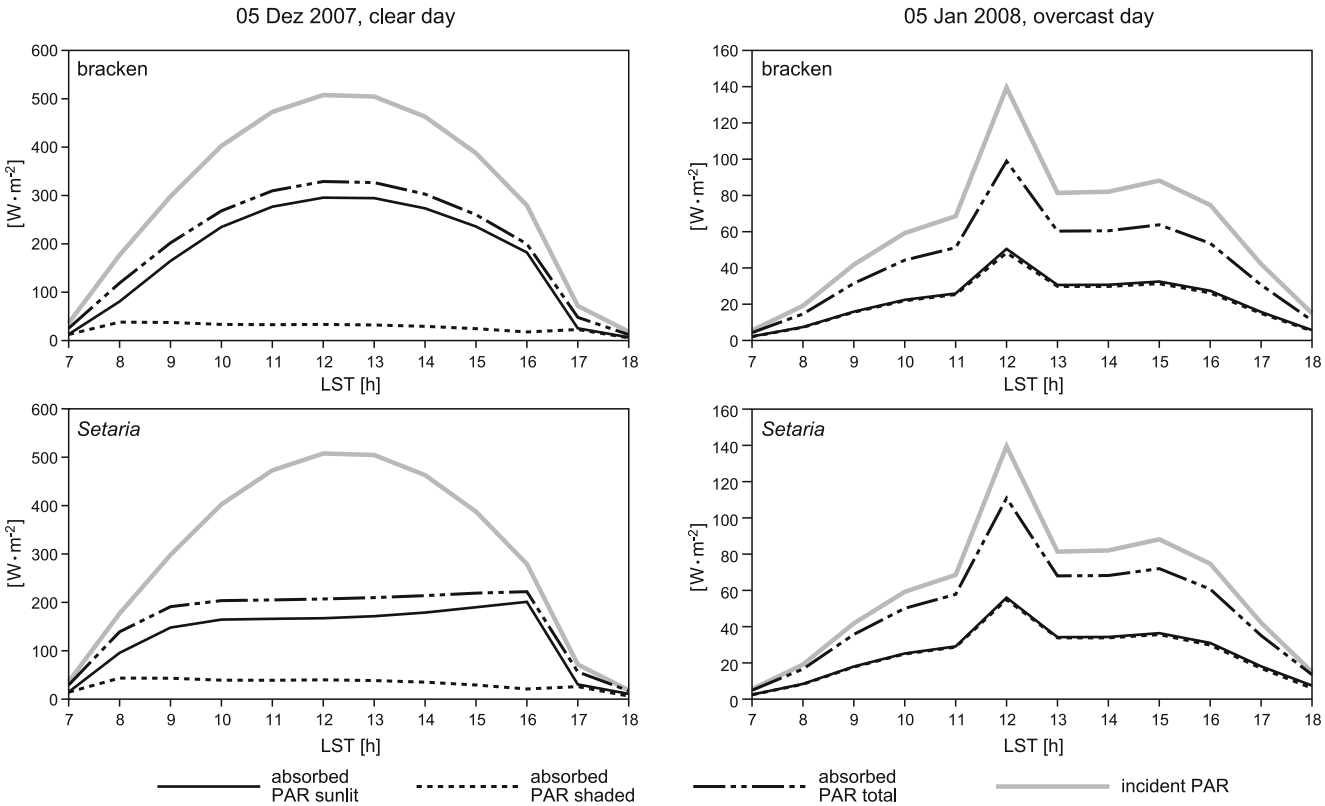

Fig. 7 PAR absorption of southern bracken and *Setaria* for (*left*) a sunny day (5 December 2007) and (*right*) a typical overcast day (5 January 2008). Radiation data for model initialization are taken from the micrometeorological station at the bracken experimental site (BS in Fig. 1)

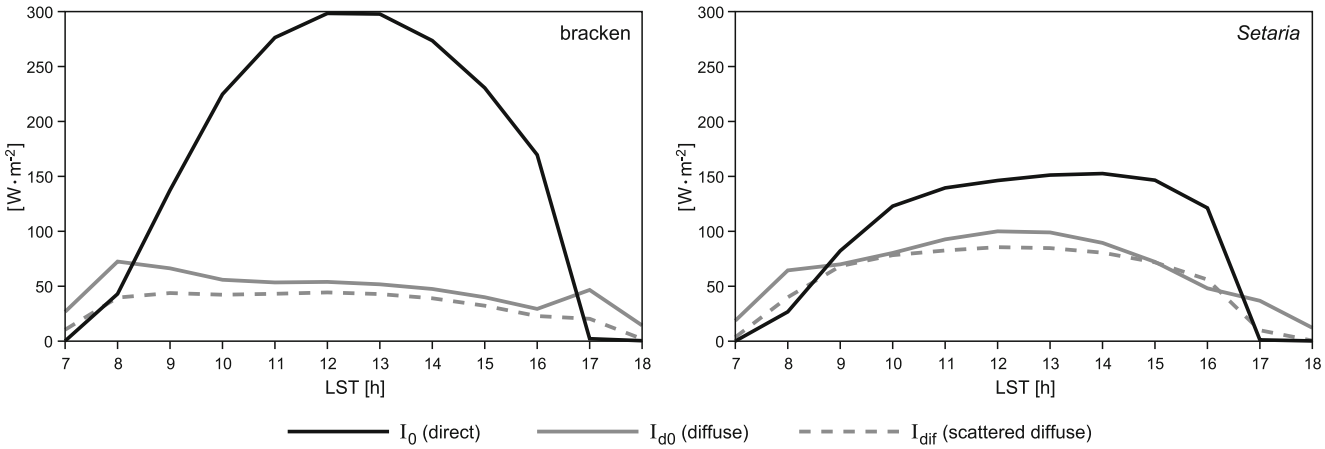

Fig. 8 Different PAR components absorbed by the sunlit canopy of bracken (*left*) and *Setaria* (*right*) on the sunny day (Fig. 7, left), 5 December 2007, based on the radiative transfer scheme

and applied to incrementally increased daily radiation maxima ranging from 100 to 1,000 W m^{-2} (with an increment of 100 W m^{-2}). Ten simulations were conducted with the radiation scheme based on the synthetically generated irradiation data. The decomposition of the generated diurnal data of global radiation by using Eqs. 4–9 leads to an increase of the direct radiation fraction with increasing radiation maximum. Consequently, a maximum of 100 W m^{-2} is mainly characterized by diffuse irradiance, a maximum of 1,000 W m^{-2} by a greater portion of direct solar irradiance. Figure 9 shows a clear species-specific distinction of PAR absorption capabilities due to varying radiation composition. Above a daily radiation maximum of about 615 W m^{-2} where direct radiation dominates, bracken absorbs clearly more direct PAR, as illustrated in Fig. 8 (direct PAR I_0). The surplus of bracken PAR absorption is continuously increasing with increasing daily radiation maximum. Below the inversion point, *Setaria* shows a slight but nearly constant advantage in PAR absorption in comparison to bracken. In a next step, the hourly radiation data at the ECSF meteorological station from 1998–2005 were evaluated to derive the frequency of daily radiation maxima in the valley. Figure 9 reveals that illumination situations favoring *Setaria* PAR absorption occur on 64% of all days, while a third of all days with more direct radiation support the higher PAR absorption potential of bracken.

By summing up the daily PAR absorption based on the synthetically generated data over all days of the year, while considering the frequency distribution of the radiation classes in Fig. 9, yields no clear advantage of one of the both species. *Setaria* absorbs 1,533 MJ m^{-2} $year^{-1}$ PAR while bracken reaches almost the same annual PAR absorption capacity of 1,530 MJ m^{-2} $year^{-1}$.

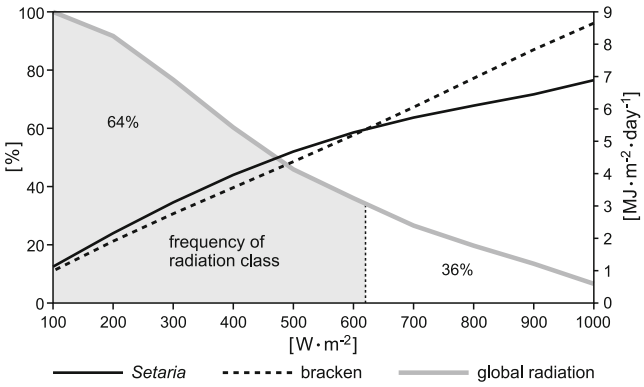

Fig. 9 Frequency and intensity of the daily irradiance maximum between 1200 and 1300 hours for the ECSF meteorological station (1998–2005) and total daily PAR absorption by bracken and *Setaria*, respectively, based on the relative diurnal course of radiation from 5 December 2007 (see Figs. 7 and 8)

Discussion

The current paper focuses on a comparison of the PAR radiation absorption capabilities of two competing species, the pasture grass *Setaria sphacelata* and an aggressive weed, the southern bracken (*Pteridium arachnoideum*), in the anthropogenic pasture system of a tropical mountain biodiversity hotspot in the eastern Andes of southern Ecuador. The work relies on a numerical two-big-leaf radiation scheme specifically parameterized with field surveys of plant functional traits, encompassing structural and optical parameters of both species. The samples selected during the field studies to derived plant functional traits were chosen to properly represent an average individual of both species under different growth situations (elevation, slope angle and aspect). The resulting structural and optical plant parameters are in good agreement with measured values for similar species or model defaults of comparable plant functional

types (as, e.g., functional group of tropical C4 grasses). The average LAI of *Setaria,* for instance, coincides quite well with default values used for climate modeling of tropical C4 grasslands with land surface models (e.g., Buermann et al. 2001). The average LAI of southern bracken is lower than the maximum values for a fully developed canopy of northern bracken (*P. aquilinum*) in England (LAI ~4 in late July; Blackburn and Pitman 1999; Pitman 2000). Leaf angle values for *Setaria* were similar to those reported by other studies of grasslands (e.g., Miller-Goodman et al. 1999; Peri et al. 2003). The average leaf angle distribution parameter for *Setaria* (χ=−0.37) is in the same order of the default value (χ= −0.3) supplied by a comparable radiation scheme for C4 grassland (Oleson et al. 2004). With regard to the optical traits, reflection measurements of bracken canopies in England (400–1,100 nm; Blackburn and Pitman 1999; *Pteridium aquilinum*) confirm the shape of the reflection curve, however with a slightly lower reflectance over the whole spectrum compared to the southern bracken. At the same time, transmittance in the visible and near infrared spectrum of bracken fronds in England (Pitman 2000) is somewhat higher in comparison to the southern bracken. Average measured PAR reflectance of *Setaria* is in almost perfect agreement with default values for C4 grasslands (11%) provided by different studies (e.g., Fisch et al. 1995) and the CLM SVAT model that uses a similar radiation scheme as described in this study (Oleson et al. 2004). Even if the overall good coincidence indicates that the gathered field samples represent an average individual under average growth conditions in the study area, it should be stressed that plant parameters used in numerical models might even change with spatial resolution. This complicates the comparison of measured plant traits and respective published model parameters. By applying the parameterized radiation scheme, it is proven that the average individuals of bracken and *Setaria* have similar PAR absorption potentials under the typical radiation conditions of the Rio San Francisco valley. Comparable similarities were also found on the landscape level by other studies (e.g., Asner et al. 1998). The most important parameter for the determination of the fraction of absorbed PAR (fAPAR) in this study where the leaf area index of the *Setaria* and bracken average individuals is differing moderately (ΔLAI=0.54 m^2m^{-2}) is the leaf–sun geometry (and thus, the leaf angle) in combination with the fractionation of the radiation components (direct, diffuse). Bracken has a higher PAR absorption potential under clear sky conditions because the leaf angle distribution favors PAR consumption during hours of high solar elevation. Similar directional effects are described for short grass ecosystem by Nouvellon et al. (2000). However, by scaling up to the landscape level, other authors stress that leaf area is the main decisive variable accounting for 60–80% of fAPAR variations while individually contributing canopy-level factors explain only a smaller proportion of fAPAR variations (Asner and Wessman 1997; Mwanamwenge et al. 1997; Asner et al. 1998).

With regard to the current state of the radiation scheme, the main uncertainty remains the decomposition of global radiation to its direct, diffuse, and PAR fractions. Comprehensive field observation of all radiation parameters is needed to derive a local decomposition function which can be implemented in the radiation scheme, replacing the currently used average decomposition functions (based on Eqs. 7–9). However, to derive local functions, several years (at least one) of diffuse radiation and PAR measurements are necessary. Most recently, respective sensors are installed and a preliminary comparison with 1 month of data and the results of the average functions used in this paper is conducted. With respect to diffuse radiation, 46% of values differ less than 10% of the calculated radiation where best results were reached for clear and completely overcast days. Generally, the average decomposition function (based on Eqs. 7–8) underestimates the observed values. With regard of PAR decomposition (based on Eq. 9), a slight overestimation by the function has been proven (+12% on average). In most situations, this counterbalances the underestimation of the composition function for diffuse radiation so that the final calculation for incoming PAR radiation provides reasonable data for the radiation scheme. Nevertheless, locally derived decomposition functions replacing Eqs. 7–9 will be implemented when sufficient data have been gathered.

The results of the simulations gained with the parameterized radiation scheme reveal that neither bracken nor *Setaria* exhibit a clear advantage in PAR absorption capability under the current climatic conditions of the study area. This means that, at first glance, PAR absorption seems not to be the reason for bracken invasion. Another reason could be that the bracken plants have more biological growth power, e.g., due to the well-developed rhizome system which make them grow faster to get more PAR, water and nutrients. However, it must be stressed that radiation absorption is just the initial factor of plant growth. The transfer of absorbed PAR to biomass depends on the radiation use efficiency (RUE) that can exhibit great species-specific variations, particularly under water and nutrient stress (for bracken, refer, e.g., to Bray 1991 and Pakeman et al. 1994; for tropical C4 grass, to Kiniry et al. 1999 and Marques da Silva and Arrabaça 2004; for PAR absorption under fertilization, to Ostrowska et al. 2008). While water is not a limiting factor in the perhumid environment of the Rio San Francisco valley, nutrient deficiency (P, N) occurs (Makeschin et al. 2008). These effects will be tested in future research by using a photosynthesis module driven by the radiation scheme presented in this paper.

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References

Antunes MAH, Walter-Shea EA, Mesarch MA (2001) Test of an extended mathematical approach to calculate maize leaf area index and leaf angle distribution. Agr For Meteorol 108:45–53

Asner GP, Wessman CA (1997) Scaling PAR absorption from the leaf to landscape level in spatially heterogeneous ecosystems. Ecol Model 103:81–97

Asner GP, Wessman CA, Archer S (1998) Scale dependence of absorption of photosynthetically active radiation in terrestrial ecosystems. Ecol Appl 8:1003–1021

Barthlott W, Hostert A, Kier G, Koper W, Kreft H, Mutke J, Rafiqpoor MD, Sommer J (2007) Geographic patterns of vascular plant diversity at continental to global scales. Erdkunde 61:305–315

Beck E, Hartig K, Roos K (2008a) Forest clearing by slash and burn. In: Beck E, Bendix J, Kottke I, Makeschin F, Mosandl R (eds) Gradients in a tropical mountain ecosystem of Ecuador. Springer, Berlin, pp 371–374

Beck E, Makeschin F, Haubrich F, Richter M, Bendix J, Valerezo C (2008b) The ecosystem (Reserva Biologíca San Francisco). In: Beck E, Bendix J, Kottke I, Makeschin F, Mosandl R (eds) Gradients in a tropical mountain ecosystem of Ecuador. Springer, Berlin, pp 1–13

Bendix J, Homeier J, Cueva Ortiz E, Emck P, Breckle S-W, Richter M, Beck E (2006a) Seasonality of weather and tree phenology in a tropical evergreen mountain rain forest. Int J Biometeorol 50:370–384

Bendix J, Rollenbeck R, Göttlicher D, Cermak J (2006b) Cloud occurrence and cloud properties in Ecuador. Climate Res 30:133–147

Bendix J, Rollenbeck R, Fabian P, Emck P, Richter M, Beck E (2008a) Temporal heterogeneities - climate variability. In: Beck E, Bendix J, Kottke I, Makeschin F, Mosandl R (eds) Gradients in a tropical mountain ecosystem of Ecuador. Springer, Berlin, pp 281–290

Bendix J, Rollenbeck R, Göttlicher D, Nauß T, Fabian P (2008b) Seasonality and diurnal pattern of very low clouds in a deeply incised valley of the eastern tropical Andes (South Ecuador) as observed by a cost-effective WebCam system. Meteorol Appl 15:281–291

Bendix J, Rollenbeck R, Richter M, Fabian P, Emck P (2008c) Gradual changes along the altitudinal gradients - the climate. In: Beck E, Bendix J, Kottke I, Makeschin F, Mosandl R (eds) Gradients in a tropical mountain ecosystem of Ecuador. Springer, Berlin, pp 63–73

Blackburn GA, Pitman JI (1999) Biophysical controls on the directional spectral reflectance properties of bracken (*Pteridium aquilinum*) canopies: results of a field experiment. Int J Remote Sens 20:2265–2282

Boland J, Scott L, Luther M (2001) Modelling the diffuse fraction of global solar radiation on a horizontal surface. Environmetrics 12:103–116

Boland JW, Ridley B, Brown B (2008) Models of diffuse solar radiation. Renew Energy 33:575–584

Boulain N, Simoni G, Gignoux J (2007) Changing scale in ecological modelling: a bottom up approach with an individual based vegetation model. Ecol Model 203:257–269

Bray JR (1991) Growth, biomass, and productivity of a bracken (*Pteridium esculentum*) infested pasture in Marlborough Sounds, New Zealand. N Z J Bot 29:169–176

Brehm G, Homeier J, Fiedler K, Kottke I, Illig J, Nöske NM, Werne FA, Breckle SW (2008) Mountain rain forest in southern Ecuador as a hotspot of biodiversity - limited knowledge and diverging pattern. In: Beck E, Bendix J, Kottke I, Makeschin F, Mosandl R (eds) Gradients in a tropical mountain ecosystem of Ecuador. Springer, Berlin, pp 15–23

Buermann W, Dong J, Zeng X, Myneni RB, Dickinson RE (2001) Evaluation of the utility of satellite-based vegetation leaf area index data for climate simulations. J Climate 14:3536–3550

Cruz DR, Reyes ES, Sánchez JS (2009) Effects of meteorological factors on airborne bracken (*Pteridium aquilinum (L.) Kuhn*.) spores in Salamanca (middle-west Spain). Int J Biometeorol doi:10.1007/s00484-009-0208-5

Dai Y, Dickinson RE, Wang Y-P (2004) A two-big-leaf model for canopy temperature, photosynthesis, and stomatal conductance. J Climate 17:2281–2299

Dickinson RE (1983) Land surface processes and climate surface albedos and energy-balance. Adv Geophys 25:305–353

Elminir HK (2007) Experimental and theoretical investigation of diffuse solar radiation: data and models quality tested for Egyptian sites. Energy 32:73–82

Erbs DG, Klein SA, Duffie JA (1982) Estimation of the diffuse-radiation fraction for hourly, daily and monthly-average global radiation. Sol Energy 28:293–302

FAO - Food and Agriculture Organisation (2001) Global forest resources assessment 2000 main report, FAO Forestry Paper 140. Rome, Italy

Fisch G, Wright IR, Bastable HG (1995) Albedo of a tropical grass: a case study of pre- and post-burning. Int J Climatol 14:103–107

Göttlicher D, Obregón A, Homeier J, Rollenbeck R, Nauß T, Bendix J (2009) Land cover classification in the Andes of southern Ecuador using Landsat ETM+data as a basis for SVAT modelling. Int J Remote Sens 30:1867–1886

Hartig K, Beck E (2003) The bracken fern (*Pteridium aquilinum*) dilemma in the Andes of South Ecuador. Ecotropica 9:3–13

Kiniry JR, Tischler CR, Van Esbroeck GA (1999) Radiation use efficiency and leaf CO_2 exchange for diverse C4 grasses. Biomass Bioenergy 17:95–112

Lappi J, Stenberg P (1998) Joint effect of angular distribution of radiation and spatial pattern of trees on radiation interception. Ecol Model 112:45–51

Larocque GR (2002) Coupling a detailed photosynthetic model with foliage distribution and light attenuation functions to compute daily gross photosynthesis in sugar maple (Acer saccharum Marsh.) stands. Ecol Model 148:213–232

Lee R, Hernández-Andrés J (2005) Colors of the daytime overcast sky. Appl Optics 44:5712–5722

Maduekwe AAL, Chendo MAC (1994) Atmospheric turbidity and the diffuse irradiance in Lagos, Nigeria, 1994. ICTP Internal report IC/IR/94/141, Trieste, Italy

Makeschin F, Haubrich F, Abiy M, Burneo JI, Klinger T (2008) Pasture management and natural soil generation. In: Beck E, Bendix J, Kottke I, Makeschin F, Mosandl R (eds) Gradients in a tropical mountain ecosystem of Ecuador. Springer, Berlin, pp 397–408

Marques da Silva J, Arrabaça MC (2004) Photosynthesis in the water-stressed C4 grass *Setaria sphacelata* is mainly limited by stomata

with both rapidly and slowly imposed water deficits. Physiol Plant 121:409–420

Marrs RH, Le Duc MG, Mitchell RJ, Goddard D, Paterson S, Pakeman RJ (2000a) The ecology of bracken: Its role in succession and implications for control. Ann Bot (Suppl B) 85:3–15

Marrs RH, Johnson SW, Le Duc MG (2000b) Control of bracken and restoration of heathland. VI. The response of bracken fronds to 18 years of continued bracken control or 6 years of control followed by recovery. J App Ecol 35:479–490

Miller-Goodman MS, Moser LE, Waller SS, Brummer JE, Reece PE (1999) Canopy analysis as a technique to characterize defoliation intensity on Sandhills range. J Range Manage 52:357–362

Mwanamwenge J, Siddique KHM, Sedgley RH (1997) Canopy development and light absorption of grain legume species in a short season Mediterranean-type environment. J Agron Crop Sci 179:1–7

Myneni BB, Asrar G, Hall FG (1992) A three-dimensional radiative transfer method for optical remote sensing of vegetated land surfaces. Remote Sens Environ 41:105–121

Nouvellon Y, Begue A, Susan Moran SM, Seen DL, Rambal S, Luquet D, Chehbouni G, Inoue Y (2000) PAR extinction in shortgrass ecosystems: effects of clumping, sky conditions and soil albedo. Agric For Meteorol 105:21–41

Oleson KW, Dai Y, Bonan G, Bosilovich M, Dickinson R, Dirmeyer P, Hoffman F, Houser P, Levis S, Niu G-Y, Thornton P, Vertenstein M, Yang Z-L, Zeng X (2004) Technical description of the Community Land Model (CLM). NCAR Technical Note NCR/TN-461+STR., Boulder, USA

Oliveira AP, Escobedo JF, Machado AJ, Soares J (2002) Correlation models of diffuse solar-radiation applied to the city of Sao Paulo, Brazil. Appl Energ 71:59–73

Ostrowska D, Pietkiewicz S, Cieslinski M, Kucinska K, Gozdowski D (2008) Biomass accumulation and absorption of photosynthetic active radiation by rapeseed plants depending on sulphur fertilization. World J Agricult Sci 4:133–136

Page CN (1986) The strategies of bracken as a permanent ecological opportunist. In: Smith RT, Taylor JA (eds) Bracken: ecology, land use and control technology. Parthenon, Carnforth, UK, pp 173–181

Pakeman RJ, Marrs RH, Jacob PJ (1994) A model of bracken (*Pteridium aquilinum*) growth and the effect of control strategies and changing climate. J Appl Ecol 31:145–154

Papaioannou G, Papanikolaou N, Retalis D (1993) Relationships of photosynthetically active radiation and shortwave irradiance. Theor Appl Climatol 48:23–27

Peri PL, Moot DJ, McNeil DL (2003) A canopy photosynthesis model to predict the dry matter production of cocksfoot pastures under varying temperature, nitrogen and water regimes. Grass Forage Sci 58:416–430

Pitman JI (2000) Absorption of photosynthetically active radiation, radiation use efficiency and spectral reflectance of bracken [*Pteridium aquilinum* (L.) Kuhn] canopies. Ann Bot 85:101–111

Pronk TE, During HJ, Schieving F (2007) Coexistence by temporal partitioning of the available light in plants with different height and leaf investments. Ecol Model 204:349–358

Richter M (2003) Using plant functional types and soil temperatures for eco-climatic interpretation in southern Ecuador. Erdkunde 57:161–181

Roderick ML, Farquhar GD, Berry SL, Noble IR (2001) On the direct effect of clouds and atmospheric particles on the productivity and structure of vegetation. Oecologia 129:21–30

Schneider LC (2004) Bracken fern invasion in southern Yucatán: a case for land-change science. Geogr Rev 94:229–241

Sellers PJ (1985) Canopy reflectance, photosynthesis and transpiration. Int J Remote Sens 6:1335–1372

Thornton PE, Zimmermann NE (2007) An improved canopy integration scheme for a land surface model with prognostic canopy structure. J Climate 20:3902–3923

Tsubo M, Walker S (2005) Relationships between photosynthetically active radiation and clearness index at Bloemfontein, South Africa. Theor Appl Climatol 80:17–25

Wang Y-P, Leuning R (1998) A two-leaf model for canopy conductance, photosynthesis and partitioning of available energy I: model description and comparison with a multi-layered model. Agr For Meteorol 91:89–111

Wang Q, Tenhunen J, Schmidt M, Kolcun O, Droesler M, Reichstein M (2006a) Estimation of total, direct and diffuse PAR under clear skies in complex alpine terrain of the National Park Berchtesgaden, Germany. Ecol Model 196:149–162

Wang X, Guo Y, Li B, Wang X, Ma Y (2006b) Evaluating a three dimensional model of diffuse photosynthetically active radiation in maize canopies. Int J Biometeorol 50:349–357

Zhang X, Zhang Y, Zhoub Y (2000) Measuring and modelling photosynthetically active radiation in Tibet Plateau during April-October. Agr For Meteorol 102:207–212

Zhang L, Moran MD, Brook JR (2001) A comparison of models to estimate in-canopy photosynthetically active radiation and their influence on canopy stomatal resistance. Atmos Environ 35:4463–4470

Record of contributions to this thesis

Chapters 1 and ***2*** including the general introduction and the synopsis of this thesis as well as the ***Summary*** (englisch and german) were written by myself.

The authors listed on the different manuscripts of Chapters 3 to 6 contributed as follows:

Chapter 3

K. Roos:	90% (concept, sample collection, allozyme electrophoresis, evaluation and statistics of all molecular analyses, interpretation of results, manuscript preparation)
A. Weig:	5% (sequencing of the cpDNA, microsatellite analysis, contribution to manuscript preparation)
E. Beck:	5% (discussion of the results, contribution to manuscript preparation)

Chapter 4

E. Beck:	30% (discussion of the results, manuscript preparation)
K. Hartig:	35% (field work, interpretation of results, contribution to manuscript preparation)
K. Roos:	35% (field work, interpretation of the results, contribution to manuscript preparation)

Chapter 5

K. Roos:	78% (concept and realisation, analysis and interpretation of the results, manuscript preparation)
R. Rollenbeck:	3% (climate data collection and processing)
T. Peters:	3% (climate data collection and processing)
J. Bendix:	6% (climate correlations, contribution to manuscript preparation)
E. Beck:	10% (discussion of the results, contribution to manuscript preparation)

Chapter 6

K. Roos:	90% (concept and realisation, analysis and interpretation of the results, manuscript preparation)
H. G. Rödel:	5% (discussion of the results in a statistical context, comments to improve the manuscript)
E. Beck:	5% (discussion of the experimental design and the results, contribution to manuscript preparation)

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Further contributors are listed in the acknowledgements of Chapters 3, 5, and 6.

List of publications

Beck E, Hartig K, **Roos K**, Preußing M & Nebel M (2008) Permanent removal of the forest: Construction of roads and power supply lines. In: Beck E, Kottke I, Makeschin F & Mosandl R (Eds): Gradients in a tropical mountain ecosystem of Ecuador. Ecological Studies 198, Springer Berlin Heidelberg, pp 361-370.

Beck E, Hartig K & **Roos K** (2008) Forest clearing by slash and burn. In: Beck E, Kottke I, Makeschin F & Mosandl R (Eds): Gradients in a tropical mountain ecosystem of Ecuador. Ecological Studies 198, Springer Berlin Heidelberg, pp 371-374.

Roos K & Beck E (2008) Regrowth of bracken (*Pteridium arachnoideum*) after burning, herbicide treatment and other weeding methods. Ecotropica 14: 76. (abstract of an oral presentation, Merian Award of the *gtö*)

Bendix J, Silva B, **Roos K**, Göttlicher D, Rollenbeck R, Nauss T & Beck E (2009) Model parameterization to simulate the PAR absorption potential of two competing plant species. International Journal of Biometeorology. DOI 10.1007/s00484-009-0279-3.

Roos K, Rollenbeck R, Peters T, Bendix J & Beck B (2010) Growth of Tropical Bracken (*Pteridium arachnoideum)*: Response to Weather Variations and Burning. Invasive Plant Science and Management. DOI: 10.1614/IPSM-D-09-00031.1 (accepted)

Roos K, Rödel HG & Beck E: Short-term and long-term effects of weed-control measures on bracken infested pastures and an attempt to regenerate abandoned pastures in South Ecuador. Weed Research (provisionally accepted, minor revision)

Roos K, Weig A & Beck E: Species composition and genetic diversity of tropical bracken invading pasture land in southern Ecuador. Molecular Ecology (submitted)

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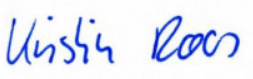
Declaration

Hiermit erkläre ich, dass ich die Arbeit selbständig verfasst und keine anderen als die von mir angegebenen Quellen und Hilfsmittel verwendet habe. Des Weiteren versichere ich, dass ich weder diese noch eine andere gleichartige Doktorprüfung an einer anderen Hochschule endgültig nicht bestanden habe.



Kristin Roos

Bayreuth, den 1. Juli 2010